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THE RELATIONS OF CARBON DIOXIDE IN ACIDIFIED BLOOD. BY T R PARSONS (*Michael Foster Student*) AND WINIFRED PARSONS (*Narrow Student of Gilton College*)

(*From the Physiological Laboratory, Cambridge*)

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It has recently been shown by Straub and Meier(1 2) that as the hydrogen ion concentration of a solution containing hæmoglobin is increased, say by means of carbonic acid, at a certain value of pH which is on the alkaline side of the iso electric point of hæmoglobin there is a sudden increase in the quantity of carbon dioxide taken up by the solution, while the reaction, as judged by the course of the CO_2 dissociation curve, remains constant. Over this range of constant pH a whole extra molecule of CO_2 is taken up for each molecule of hæmoglobin present. The original discoverers of this phenomenon explain it as being due to the direct combination of this extra molecule of CO_2 with the hæmoglobin molecule, but direct measurements of the hydrogen ion concentration over this range(3) have shown that the more probable explanation is that each hæmoglobin molecule is giving off a single atom of "available" sodium to form sodium bicarbonate with the extra carbon dioxide. Straub and Meier(2) have also found evidence of the combination of a second similar extra molecule of CO_2 at a more acid reaction. These facts suggest several possibilities, but in the first place it is clear that further evidence on the question as to the mode of combination of these extra molecules of CO_2 can be obtained from the results of experiments on the distribution of the CO_2 between the corpuscles and plasma of blood over a range of pH including that at which the extra gas is taken up. For if the extra CO_2 is combined directly with the hæmoglobin in a non ionising compound, it must all be confined to the corpuscles, while if it is combined as sodium bicarbonate then this salt, first formed in the corpuscles according to our theory, will distribute

itself between the two phases by diffusion and ionic interchange. The concentrations of sodium bicarbonate inside and outside the corpuscular membrane will not necessarily be equal, but one can be certain that at least that portion of the extra CO_2 which is found in the plasma cannot be combined with the hæmoglobin, but is probably in the form of sodium bicarbonate. It has been the object of the present series of experiments to obtain evidence on this point.

I. *Methods.*

For our purpose it was necessary to saturate acidified blood with gas mixtures containing various tensions of CO_2 and then to separate the plasma without loss of gas and to estimate its carbon dioxide content. In all cases we used human blood drawn in sufficient amount by free puncture of the finger at the root of the nail, and received into a dish dusted with finely powdered neutral potassium oxalate to prevent clotting. The blood was acidified by the addition of the required small measured volume of concentrated lactic acid of sp. gr. 1.2, but in order to avoid damage to the corpuscles the acid was not added directly to the blood; it was first diluted and partially neutralised by means of a small quantity of plasma obtained by the settling or gentle centrifuging of the measured portion of blood. By the adoption of this method the laking of the red cells was practically entirely prevented, so that, as a rule, the plasma separated at once from this acidified blood showed no tinge of colour indicative of the presence of free hæmoglobin. But in spite of the fact that the stock of acidified blood was always kept on ice during a series of experiments, it was found that a small but progressive amount of hæmolysis occurred on standing, which made it necessary to carry out the observations on any particular mixture as quickly as possible after the acidification, and always on the same day. Furthermore, in order to avoid the effects of any regular change due to hæmolysis the experiments were always carried out in an arbitrary and not in a regular order with regard to CO_2 tensions. The small quantities of lactic acid required for these experiments—usually of the order of $\frac{1}{20}$ th of a c.c.—were measured as accurately as possible from a capillary pipette. This measurement was obviously liable to considerable experimental error—not that this is of importance for our present purpose, but it should be mentioned as the chief reason for the lack of exact correspondence in quantitative behaviour of the various blood-lactic acid mixtures of ostensibly the same composition but made up at different times. In each case the CO_2 gas mixture was made up in air,

so that the blood investigated was always practically completely saturated with oxygen. It is intended to extend this investigation later to a study of the corresponding properties of reduced blood.

The saturating arrangements were similar to those previously described(4), when equilibrium had been established a sample of the gas mixture was transferred to a Haldane's apparatus for analysis for CO_2 , and then the blood was transferred to the special centrifuge tube which up to this moment had been connected in series with the saturator in the thermostat bath. The separation of corpuscles and plasma was carried out in the closed tube figured in a previous communication(5) by means of a directly driven electric centrifuge enclosed in an electrically heated and controlled air thermostat working at the same temperature as that of the saturating bath. Owing to the circumstance that the total volume of blood used in each experiment was only about 1 c.c., it was found to be advisable to protect the resulting half c.c. or so of plasma from loss of CO_2 during the measuring off for analysis by allowing it to come into contact only with the gas mixture with which it had already been brought into equilibrium. The device used for this purpose is shown in Fig. 1. When the separation was finished and the plasma was to be withdrawn a short length of glass tubing was inserted by a narrowed end into the end of the rubber tube closing the centrifuging vessel. This glass tube was closed at the top by means of a small thick plug of cotton wool through which were inserted the long capillary point of the plasma measuring pipette and also a capillary glass tube conveying a stream of the gas mixture from its storage bottle. When the small volume of air in the upper part of the apparatus had been expelled through the cotton wool, the clip was opened and the point of the pipette lowered so as to suck off the plasma as completely as possible. A measured volume of this plasma was then allowed to form a layer below the surface of 2 c.c. of N/40 baryta solution in one bottle of a Barcroft differential apparatus in which the carbon dioxide content was measured by treatment with acid in the usual way. Lactic acid was the acid used. The apparatus had been previously calibrated by liberating in it under similar conditions

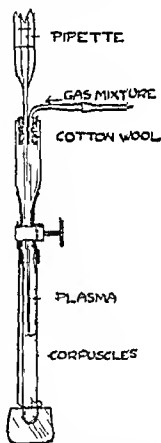


Fig. 1 Apparatus for withdrawing plasma from centrifuge tube without exposure to air

known volumes of CO_2 from a standard solution of pure sodium carbonate. It was soon found to be much safer to suck up the plasma by slowly opening a clip compressing a small indiarubber ball connected by rubber tubing to the top of the pipette. But in order to keep the drainage error constant the plasma was allowed always to flow out by gravity by opening a tap which placed the cavity of the indiarubber ball in communication with the atmosphere. During the withdrawal of the plasma the centrifuge tube was kept immersed in a beaker of water at the temperature at which the saturating and centrifuging had taken place. It will be seen that in this way throughout the whole process from the saturating of the blood to the measuring off of the plasma the liquid was kept at a constant known temperature and was in contact only with the gas mixture with which it was desired that it should be in equilibrium.

In order to determine the magnitude of the experimental error involved in our measurements we carried out two series of control experiments. In the first of these we used portions of the same solution of sodium carbonate, measured with great care so as to maintain a constant drainage error, and obtained values of 244.6, 244.1, 243.3 c.c. per 100 c.c. as a result of successive determinations of its total CO_2 content. Our second, and more elaborate, test consisted in taking a mixture of blood and lactic acid such as we used for most of our experiments, and in making a series of determinations throughout the course of a day of the CO_2 content of the plasma obtained from successive samples after these had been brought into equilibrium with the same gas mixture. The details and results of these measurements are given in Table I.

TABLE I. Successive determinations of the total CO_2 content of the plasma from a mixture of 10 c.c. oxalated blood + .04 c.c. syrupy lactic acid. The saturation and centrifuging were both carried out at 15°C .

Time of commencement of exp.		Tension of CO_2 in mm. Hg.	Total CO_2 content of plasma (c.c. per 100 c.c.)
h.	m.		
12	15	212.0	70.1
2	9	211.9	72.8
3	57	211.9	70.0
5	45	213.0	68.4
8	50	213.3	71.4

The divergencies between these results indicate the maximum total effect of all the possible sources of error in our results, including such factors as possible changes in the properties of the acidified blood on being kept, any slight loss of gas during centrifuging or measuring, and so on.

II. Experiments.

With this technique we proceeded first of all to determine whether the combination of the first "extra" molecule of CO_2 produced an inflection in the curve showing the relation between CO_2 tension and content in the plasma similar to that described by Straub and Meier in the whole blood. A sample of 12.5 c.c. of ovalated human blood was acidified with .05 c.c. of syrupy lactic acid of sp gr. 1.2, saturated with mixtures of air and CO_2 at suitable tensions and then centrifuged at body temperature. The total CO_2 content of the plasma was determined as above described, and the results are recorded in Table II. We have plotted these values in Fig. 2 in a diagram which

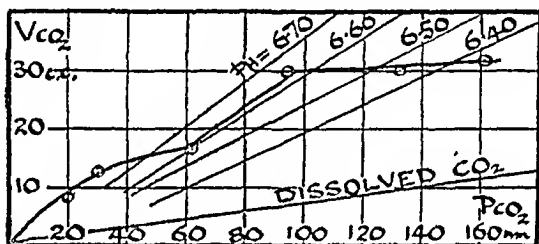


Fig. 2 CO_2 content of the "true plasma" from acidified oxygenated blood at various CO_2 tensions Temp. 38°C .

TABLE II. The total CO_2 content of the plasma of acidified blood at various CO_2 tensions. The saturation and centrifuging were carried out at 38°C .

Tension of CO_2 in mm Hg	CO_2 content of plasma cc per 100 cc)
20.2	8.21
30.2	12.4
62.5	16.4
94.1	29.9
132.3	30.0
161.5	31.5

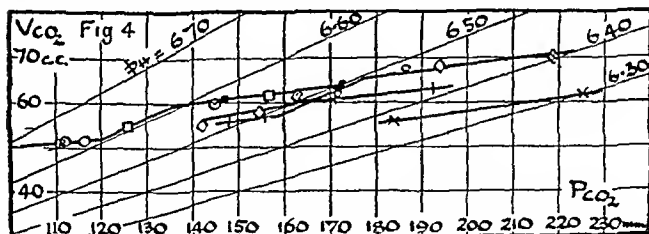
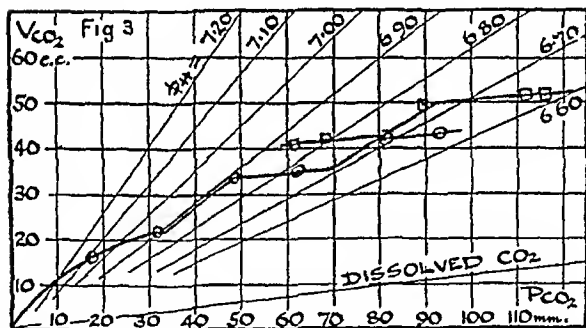
includes the lines of constant $p\text{H}$ in bicarbonate solutions at body temperature calculated according to Hasselbalch's data(6), and also the line representing the physical solubility of CO_2 in plasma. It will be seen that the extra portion of CO_2 which is taken up is distributed between corpuscles and plasma, with the result that an inflection occurs on the true plasma CO_2 curve similar to that already described by Straub and Meier in whole blood(1). The extra CO_2 was therefore in

a diffusible form, presumably HCO_3 ions, and not combined directly with the hæmoglobin. Furthermore, it is seen that the curve we have obtained follows the line of constant $p\text{H}$ of about 6.61, the reaction remaining the same throughout the inflection. Straub and Meier give the position of the first inflection at body temperature in whole blood acidified with hydrochloric acid as $p\text{H} = 6.54$. The difference between their value and the one we have obtained in this way for the plasma depends on the circumstance that Straub and Meier calculate their values of $p\text{H}$ from the CO_2 dissociation curves of whole blood, whereas here we have dealt with the plasma alone. We have already shown⁽⁴⁾ that the value for the $p\text{H}$ of the whole blood of the same subject as was used in this experiment, as measured electrometrically, differed by .07 from the value obtained by calculation by means of Hasselbalch's formula, and have pointed out that when whole blood is used in an electrometric determination it is really the reaction of the plasma or serum which is so measured. Joffe and Poulton⁽⁷⁾ have also brought forward confirmatory evidence on this point. Thus the difference of .07 between the $p\text{H}$ at which the first inflection appears in whole blood and in plasma is readily accounted for. It is also to be observed that the total range over which the reaction remains constant corresponds to the taking up of about 11 c.c. of extra combined CO_2 per 100 c.c. of plasma. If one molecule of extra CO_2 were taken up for each molecule of hæmoglobin present in solution, and if the blood contained a normal concentration of hæmoglobin, the volume of extra CO_2 taken up in the whole blood would be 18.5 c.c. Whether or not the difference between this value and the one which we have actually observed in the plasma is to be accounted for in terms of an unequal distribution of the CO_2 between the corpuscles and the surrounding fluid or whether the lactic acid had destroyed a certain proportion of the hæmoglobin we cannot decide. Straub and Meier⁽¹⁾ found that in whole blood acidified with hydrochloric acid the size of the first inflection (at a $p\text{H}$ of 6.54) corresponded to a taking up of about 10 c.c. of extra combined CO_2 .

Our next experiments give information with regard to the effect of temperature on the reaction at which this first inflection occurs. A similar mixture of the same blood and lactic acid was saturated with air. CO_2 mixtures at 15°C ., and also centrifuged at room temperature, instead of at body temperature. The results of the determinations of the CO_2 contents of the true plasma at various CO_2 tensions are recorded in Table III A and plotted (with others) in Fig. 3.

TABLE III The total CO_2 content of the plasma of acidified blood at various CO_2 tensions
The saturation and centrifuging were carried out at 15°C

Tension of CO_2 in mm. Hg		CO_2 content of plasma (c.c. per 100 c.c.)	Tension of CO_2 in mm. Hg		CO_2 content of plasma (c.c. per 100 c.c.)
A	17.2	16.2	D.	146.9	60.4
	31.9	21.9		172.4	63.0
	48.5	23.8			
	62.0	24.9		171.2	60.8
	81.9	41.8		186.3	67.6
	93.1	43.3			
B	61.5	40.8		184.0	55.9
	68.6	41.9		225.2	61.9
	89.5	49.2			
	111.8	51.5		148.3	55.4
	116.0	51.1		155.8	56.6
	141.7	50.7		171.0	60.5
C				192.3	62.9
	110.8	50.7			
	125.7	44.7		142.0	54.9
	156.9	61.2		154.8	57.8
				162.9	61.4
				193.7	67.9
				218.6	61.9



Figs. 3, 4 CO_2 content of the true plasma from acidified oxygenated blood at various CO_2 tensions Temp. 15°C

Here again we find a well-marked inflection in the true plasma curve, but now at a pH of about 6.88 instead of at 6.61 as at $38^{\circ} C$. It should be noted that in drawing the lines of constant pH ("isohydrics") in Figs. 2 and 3 due account has been taken of the effect of temperature on the bicarbonate-carbonic acid equilibrium. We conclude then that change of temperature produces a marked change in the hydrogen-ion concentration at which this inflection occurs. We shall refer to this result again later (p. 16). For the moment we shall merely point out that the size of this inflection corresponds to the taking up of about 9 c.c. of combined CO_2 ; it is therefore slightly smaller than the corresponding inflection at body temperature.

It will be remembered that Straub and Meier(2) described the occurrence of a second inflection in the CO_2 dissociation curve of hæmolyzed acidified blood. The determinations at the higher CO_2 tensions recorded in Table III A and Fig. 3 suggest that a similar second inflection occurs also in the plasma curves which we have determined, in this case at a constant value of pH of about 6.73. The occurrence of this second inflection was confirmed by a further series of determinations on a similar mixture of blood and lactic acid, the results being recorded in Table III B and in Fig. 3. The size of the inflection in this case is markedly smaller, corresponding to an increase of CO_2 content of only about 7 c.c. p.c.

We next continued the investigation at still higher pressures of CO_2 in order to determine whether there were any further sudden changes in CO_2 combining power. There seemed no reason for supposing that there should be only two of these, although Straub and Meier regard the existence of *two* such changes as an important factor in their explanation of the phenomenon in terms of the colloidal properties of hæmoglobin. Indeed, the last determination recorded in Table III B suggests the possibility of the occurrence of a third inflection, and a further series of determinations on another similar mixture of blood and lactic acid confirms the suggestion. The values are given in Table III C and are included among those plotted in Fig. 4. We are fortunate in this case in having succeeded in mixing the blood and acid in exactly the same proportions as occurred in the mixture which gave the results recorded in Table III B so that the curve in Fig. 4 may be regarded as an exact continuation of that in Fig. 3. It will be seen that the inflection occurs at a pH of about 6.61 and that the range of constant reaction corresponds to an increase of *combined* CO_2 of only about 6 c.c. p.c.

So far, then, we have shown the existence, in this "true plasma"

CO₂ dissociation curve, of three sudden increases of CO₂ combining power at values of *pH* of 6.88, 6.73 and 6.61 respectively. It will be seen at once that there is practically the same interval of *pH* between the first and second of these values as between the second and third. This relation suggests that any possible further inflection would probably occur at an equal interval of *pH* beyond that last described. As the isohydric lines in this region of the curve are not very different in slope from the line indicating the physical solubility of CO₂ it was necessary to carry out a number of determinations in order to be certain of the course of the curve, but the results recorded in Table III D and in Fig. 4 seem to indicate a definite but small inflection in the expected position, namely at a *pH* of about 6.48. It corresponds to an increase of CO₂ combining power of only about three volumes per cent. This seems to be the last of these sudden increases in CO₂ carrying power, for measurements carried out at a still more acid range of reaction showed no sign of an inflection, but, on plotting, gave a straight line parallel to that representing the physical solubility of CO₂ (see Table IV and Fig. 5).

TABLE IV The total CO₂ content of the plasma of acidified blood at high CO₂ tensions. The saturation and centrifuging were carried out at 15° C.

Tension of CO ₂ in mm. Hg	CO ₂ content of plasma (cc. per 100 cc.)
217.7	71.8
239.1	75.6
258.5	77.6
291.9	83.7
302.9	86.0
307.5 (?)	87.9

Lastly, in order to make certain that these sudden increases in the CO₂ combining power of the plasma were due to changes in the composition of the corpuscles and consequent shifting of the ionic equilibrium between corpuscles and plasma, we determined the CO₂ dissociation curve of a sample of "separated" plasma. 17.5 cc. of oxalated human blood were acidified, in the manner previously described, with 0.7 cc. of syrupy lactic acid. The mixture was centrifuged at room temperature without special precautions against loss of CO₂, and the dissociation curve of the resulting plasma was determined at room temperature. The results are recorded in Table V, and also represented in Fig. 5, from which it will be seen at once that the curve is perfectly smooth and shows no sign of a sudden change of direction at any point.

TABLE V. The CO_2 dissociation curve of the separated plasma from a mixture of 17.5 c.c. of oxalated human blood and .07 c.c. of syrupy lactic acid. Temp. 15°C .

Tension of CO_2 in mm. Hg.	CO_2 content of plasma (c.c. per 100 c.c.)
12.4	18.5
26.1	23.1
58.5	30.3
107.1	38.4
152.9	42.4
195.0	50.0

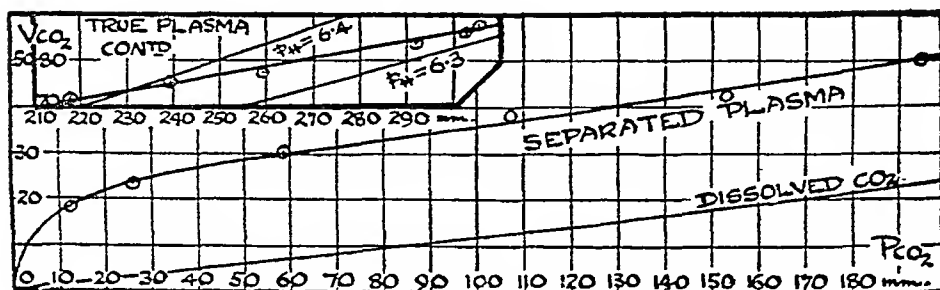
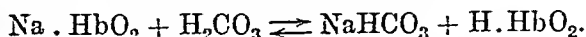


Fig. 5. The CO_2 dissociation curve of the "separated plasma" from acidified oxygenated blood. Inset—continuation of the "true plasma" curve at high CO_2 tensions.

III. Discussion.

The results here recorded show, then, that the sudden increase of CO_2 combining power observed by Straub and Meier in whole acidified blood at a certain reaction is not confined to the corpuscles but is also shown by the plasma. There is thus a sudden inflection in the true plasma CO_2 dissociation curve at a particular value of pH. This fact, together with the observation that the CO_2 dissociation curve of the "separated plasma" shows no such inflection, proves that the extra CO_2 taken up by the whole blood over this range is first taken up by the corpuscles, and there forms some compound, for example, sodium bicarbonate or the HCO_3 ion, capable of diffusing out into the plasma. This is readily explained in terms of the theory that during the inflection the hæmoglobin is not combining directly with CO_2 , but is furnishing "available" sodium according to the equation:



One would suppose that the first inflection represents the giving off of one atom of available sodium from each molecule of hæmoglobin. The second inflection is due to the giving off of another atom. The initial curved portion of the line at the lowest

CO_2 tensions is taken to represent, also, the formation of a molecule of sodium bicarbonate from sodium which, for reasons to be discussed later, is given off only gradually from the hæmoglobin and not at constant reaction.

There now arises the question of the explanation of the third and fourth inflections. In the first place it should be pointed out that the existence of these third and fourth inflections necessitates some modification of the views put forward by previous writers as to the nature of the first two sudden changes. Straub and Meier(2), who discovered these first two inflections in the CO_2 dissociation curve of acidified blood, suggested that each is due to a sudden change in the charge carried by the colloidal hæmoglobin: the hæmoglobin is supposed to lose its negative charge at a pH of about 7 and to become capable of combining directly with CO_2 (so giving rise to the first inflection). When it further acquires a positive charge, at a pH of 6.53, it is supposed to become capable of taking up a further molecule of CO_2 , so producing the second inflection. It is to be noted that these values were obtained for hæmolysed blood—the corresponding ones for whole blood are different, as already described on p. 6. It will be seen that the first sudden change of charge occurs at a pH on the alkaline side of the isoelectric point of oxyhæmoglobin ($\text{pH} = 6.74$, Michaelis(8), p. 57), and that the second occurs on the acid side, so that, apart from the necessity of explaining the relation between the charge carried by the hæmoglobin and its direct combining power for CO_2 , it is evident that this theory as it stands admits of the possibility of the occurrence of only two such inflections.

On the other hand, Michaelis(9) refuses to regard these inflections as lines of constant reaction, and, disregarding the existence of the second inflection described by Straub and Meier, assumes that the first inflection actually extends over a range of reaction such that when it ends, the actual reaction in the mixture is on the acid side of the isoelectric point. The fact that the inflection occurs in the plasma is evidence, we think, against this interpretation, and is in favour of the view that by some means the hæmoglobin is keeping the reaction of the blood constant over this range. Further, we have made a few direct measurements of the reaction changes occurring under these conditions(3), and, so far as our measurements have gone, have succeeded in confirming Straub and Meier's contention that the inflections in the CO_2 dissociation curves are truly regions of constant reaction. But these considerations do not help us to arrive at the explanation of the last two

inflections. The difficulty is that while the first two inflections occur at reactions on the alkaline side of the isoelectric point of oxyhæmoglobin, and thus can be explained by the giving up of sodium atoms from the hæmoglobin molecules, the last two occur when the reaction of the plasma is more acid than that corresponding to the isoelectric point of oxyhæmoglobin, so that this substance might be supposed under these circumstances to be acting as a base.

In the absence of direct determinations of the reaction in the interior of the corpuscle two possibilities, then, exist. On the one hand it is possible that the reaction *inside* the cell is still on the alkaline side of the isoelectric point of oxyhæmoglobin—that the permeability of the corpuscle membrane for hydrogen-ions, and also for the other ions which influence the C_H , changes as the CO_2 tension (and with it the C_H) increases in such a way as to reverse the relation between the C_H in corpuscle and plasma obtaining when the C_H in the plasma is smaller. The reasons for believing that when the reaction of the plasma is comparatively alkaline the contents of the red cells have a higher C_H than that of the plasma have been dealt with in our previous works (4, 10, 11): if at high hydrogen-ion concentrations of the plasma this relation is reversed we should have a phenomenon very similar to that described for the effect of reaction on the distribution of chlorine ions across a membrane by Rona and György (12). The reaction obtaining inside the corpuscle during the third and fourth inflections might then be still on the alkaline side of the isoelectric point of oxyhæmoglobin, and these inflections could then be interpreted as representing the splitting off of yet two more atoms of sodium from the oxyhæmoglobin molecule, making a total of five sodium atoms per molecule of hæmoglobin. This explanation is supported by the results of analysis of the CO_2 dissociation curve of blood and by the results of blood ash analysis (13). But the possibility is not excluded that the reaction of the corpuscle contents may follow that of the plasma quite regularly in such a way that at all values of pH of the plasma the reaction of the corpuscle contents is more acid than that of the plasma. In this case the reaction of the corpuscle contents during the third and fourth inflections will be on the acid side of the isoelectric point of oxyhæmoglobin, so that this substance will be no longer acting as an acid but as a base. As such it will presumably be able to combine with carbonic acid forming a carbonate: the presence of the third and fourth inflections in the true plasma CO_2 dissociation curve of this acidified blood is then to be explained by the ionisation of this hæmoglobin bicarbonate with the production of HCO_3 ions which

are capable of exchange through the corpuscle membrane with the chlorine ions of the plasma. Some support for this view is given by the results of a few experiments in which we have determined not only the CO_2 content of the plasma (Table VI) but also that of the whole blood

TABLE VI. Distribution of CO_2 between corpuscles and plasma of acidified blood
Temp 15°C

Tension of CO_2 in mm. Hg	Total CO_2 content (c c per 100 c c)	
	of whole blood	of plasma
146.9	55.9	60.4)
172.4	119.7 (?)	63.0)
171.2	57.8	60.8)
186.3	63.9	67.6)
198.2	50.4	62.1)
169.1	50.1	56.1)
189.1	52.8	65.1)
153.6	50.1	57.9
185.8	57.4)	—
127.7	45.2	—
156.1	53.4)	—

This was easily done by taking an extra portion of the blood in the saturator and arranging a small straight glass tube between the centrifuging tube and the tube leading to the gas analysis apparatus. On transferring the blood, the centrifuging tube is first filled and then the excess of blood is allowed to pass over into the straight tube which can be clipped off at each end as soon as it is filled. The blood was withdrawn from this by the same pipette as was used for the plasma determinations and analysed in the same differential apparatus. It will be seen that even in this very acid blood the total CO_2 concentration in the whole blood is always less than that in the plasma, so that the total CO_2 concentration in the corpuscle contents is also less than that in the plasma. Now, even if all the combined CO_2 in the corpuscles were in the form of NaHCO_3 , this would mean that the corpuscular contents had a greater hydrogen-ion concentration than the surrounding plasma, and, if some of the combined CO_2 were in a form not completely dissociated into HCO_3 ions, the acidity would be all the greater. But on the other hand this argument assumes that there is unrestricted diffusion of dissolved CO_2 into the corpuscle so that the tension of CO_2 inside the corpuscle is the same as outside: on this point there seem to be no data at all and also in judging the concentration of combined CO_2 in the corpuscle it is assumed that the whole corpuscle is composed of the aqueous phase, while the effective volume is almost certainly less than this on account of the space occupied by the colloidal constituents of the corpuscle. In other words,

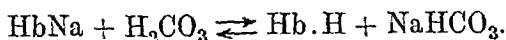
inflections. The difficulty is that while the first two inflections occur at reactions on the alkaline side of the isoelectric point of oxyhæmoglobin, and thus can be explained by the giving up of sodium atoms from the hæmoglobin molecules, the last two occur when the reaction of the plasma is more acid than that corresponding to the isoelectric point of oxyhæmoglobin, so that this substance might be supposed under these circumstances to be acting as a base.

In the absence of direct determinations of the reaction in the interior of the corpuscle two possibilities, then, exist. On the one hand it is possible that the reaction *inside* the cell is still on the alkaline side of the isoelectric point of oxyhæmoglobin—that the permeability of the corpuscle membrane for hydrogen-ions, and also for the other ions which influence the C_H , changes as the CO_2 tension (and with it the C_H) increases in such a way as to reverse the relation between the C_H in corpuscle and plasma obtaining when the C_H in the plasma is smaller. The reasons for believing that when the reaction of the plasma is comparatively alkaline the contents of the red cells have a higher C_H than that of the plasma have been dealt with in our previous works(4, 10, 11): if at high hydrogen-ion concentrations of the plasma this relation is reversed we should have a phenomenon very similar to that described for the effect of reaction on the distribution of chlorine ions across a membrane by Rona and György(12). The reaction obtaining inside the corpuscle during the third and fourth inflections might then be still on the alkaline side of the isoelectric point of oxyhæmoglobin, and these inflections could then be interpreted as representing the splitting off of yet two more atoms of sodium from the oxyhæmoglobin molecule, making a total of five sodium atoms per molecule of hæmoglobin. This explanation is supported by the results of analysis of the CO_2 dissociation curve of blood and by the results of blood ash analysis(13). But the possibility is not excluded that the reaction of the corpuscle contents may follow that of the plasma quite regularly in such a way that at all values of pH of the plasma the reaction of the corpuscle contents is more acid than that of the plasma. In this case the reaction of the corpuscle contents during the third and fourth inflections will be on the acid side of the isoelectric point of oxyhæmoglobin, so that this substance will be no longer acting as an acid but as a base. As such it will presumably be able to combine with carbonic acid forming a carbonate: the presence of the third and fourth inflections in the true plasma CO_2 dissociation curve of this ^{acidified} blood is then to be explained by the ionisation of this hæmoglobin bicarbonate with the production of HCO_3 ions which

other words, if we regard oxyhæmoglobin as a fairly strong acid in an alkaline solution, we must regard it as being a similarly strong base in acid solution, so that under these conditions it would be expected to form well ionising bicarbonate (cp. also Michaelis⁽ⁿ⁾). Naturally the ionisation of this bicarbonate will be depressed by the HCO_3 ions already present from the sodium bicarbonate previously formed at lower CO_2 tensions. Then, secondly, the lactic acid with which we originally acidified the blood will have reduced the CO_2 carrying power of the fluid very considerably, and it is not impossible that this effect of the lactic acid should show itself over one range of reaction more than over another.

We have not yet dealt with the reasons why the inflections should be so sudden and how it is that they follow the straight lines of constant reaction. The clue to this question was furnished by a chance observation which we made during the course of our experiments. Having exposed an acidified blood mixture to a high CO_2 tension, we happened to dilute some of the fluid with water. As would be expected, hæmolysis quickly occurred, but it was seen that the hæmoglobin was not in solution, but had been precipitated in the acid solution. On the other hand, dilution of some of the original lactic acid-blood mixture, not treated with CO_2 , led to hæmolysis, but no precipitation of the hæmoglobin. This seems to show that at a certain CO_2 tension the reaction of the corpuscle contents will be sufficiently acid to precipitate the hæmoglobin, and that at tensions of CO_2 above this the hæmoglobin will be always in the solid condition. Now, while the hæmoglobin is in solution, it conforms to the behaviour of other electrolytes; it does not form a separate phase in the system and the law of mass action may be applied to it. This is shown by the way in which the law of mass action can be used to explain the CO_2 dissociation curves and the pH curves of normal blood. But as soon as the hæmoglobin is in the precipitated condition it does form an additional phase in the system, so that the number of degrees of freedom is one less than before. In other words the conditions in a system composed of CO_2 and hæmoglobin and hydrogen-ions are similar to those obtaining in the system CaO , CaCO_3 and CO_2 , and just as in this latter system, at a given temperature, there is a certain fixed pressure of CO_2 below which all the CaCO_3 is decomposed and above which it is all stable, so in the hæmoglobin system there is a certain hydrogen-ion concentration below which all the sodium is combined with hæmoglobin and above which it is all combined with CO_2 to form NaHCO_3 .

If we represent the equilibrium in the usual way:



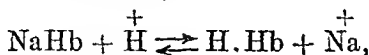
Applying the law of mass action, we have:

$$\frac{[\text{Hb.Na}][\text{H}_2\text{CO}_3]}{[\text{Hb.H}][\text{NaHCO}_3]} = K,$$

where the concentration of each reacting molecule is represented by its formula enclosed in square brackets, and K is the equilibrium constant.

But while solid Hb.Na and Hb.H are present in the system, the concentrations of these substances will be constant, being determined by their respective solubilities, so that the ratio $\frac{[\text{H}_2\text{CO}_3]}{[\text{NaHCO}_3]}$ will also be equal to another constant, K_1 , say; and seeing that it is this ratio which determines the hydrogen-ion concentration in the solution, the reaction will remain constant over this range.

These considerations are of fundamental importance for the theory of chemical systems in which colloids take part in the equilibrium. We have here a colloid behaving in the same way as a crystalloid so long as it remains in solution, and only forming a separate phase as soon as precipitation occurs. On account of this circumstance—that the hæmoglobin seems to be mainly in the precipitated condition during the inflections—we cannot draw any certain conclusions as to the effect of temperature on the dissociation constants of hæmoglobin. We have already pointed out that the value of $p\text{H}$ at which the *second* sodium atom is dissociated from the hæmoglobin molecule is 6.62 at 38° C., while at 15° C. it is more alkaline, namely 6.88. If we represent the reaction in a slightly different way, thus:



we may say that at the higher temperature the hæmoglobin holds more firmly to its available sodium; but this result may be explained as due either to a change in the equilibrium constant of this reaction, *i.e.* to a change in the strength of hæmoglobin as an acid, or to a change in the relative solubilities, and so of the relative concentrations, of the sodium salt and of the free acid.

Although the investigations we have recorded were carried out on blood which was so heavily acidified as to produce reactions far more acid than those which could ever occur normally—or even pathologically—in the body, yet the results are not devoid of significance in connection with the normal physiology of the blood. In the present experiments, while the hæmoglobin remains in solution, it obeys the ordinary law of

mass action, and gives off its "available" sodium gradually, but it becomes precipitated at a certain reaction, forms a separate phase in the system, and thenceforward gives off its "available" sodium by sudden steps at fixed hydrogen-ion concentrations. But, in the normal blood, the conditions are such that the *whole* of the "available" sodium is given off gradually, approximately according to the law of mass action, as the CO_2 tension increases. There is therefore in normal blood some mechanism which keeps the hæmoglobin in solution while it is robbed by carbonic acid of its available base. That the permeability relations of the red corpuscles form the fundamental factors in the working of the mechanism cannot well be doubted, but the complete nature of the forces at work has yet to be demonstrated.

Then again, seeing that we may regard the hæmoglobin as an electrolyte in true solution, it follows that a certain amount of it—varying with the conditions of its environment—will exist in the form of hæmoglobin ions. This being so, the question at once suggests itself—has the ionic condition of the hæmoglobin any significance with relation to its carrying power for oxygen? L. J. Henderson⁽¹¹⁾ has brought forward reasons for the view that the sodium salt of hæmoglobin has a greater affinity for oxygen than the free acid, Rona and Ylppo⁽¹²⁾ have shown that at a given oxygen tension the oxygen combining power of hæmoglobin is less at reactions in the neighbourhood of the isoelectric point than at reactions either more acid or more alkaline than this. Might we not correlate these observations by assuming that the charged ions of hæmoglobin have a greater oxygen combining power than the uncharged molecules, and that the sodium salt of hæmoglobin is much more completely ionised than the free acid? The possibility of the occurrence of ionisation in hæmoglobin solutions has been already pointed out by Roaf⁽¹³⁾, and the observations we have recorded give a further hint as to the complexity of hæmoglobin solutions. For not only must we assume the presence both of molecular aggregates and of ions, but the possibility of the formation of ions of various valencies must be realised, for our experiments have indicated that hæmoglobin is to be regarded not as a simple univalent, but as a polyvalent colloidal ampholyte.

SUMMARY.

(1) There are four sudden inflections in the carbon dioxide dissociation curve of the "true" plasma of acidified oxygenated blood. At a temperature of 15°C . these occur at pH 's of 6.88, 6.73, 6.61 and 6.18 respectively. At 38°C . the pH at which the first inflection occurs is 6.61.

(2) The first two of these inflections may probably be explained as due to the formation of sodium bicarbonate from sodium obtained from the oxyhæmoglobin molecule: the remaining inflections may be due to a similar formation of sodium bicarbonate, or to the formation of a bicarbonate of oxyhæmoglobin. But if such an oxyhæmoglobin bicarbonate is formed it must necessarily undergo ionisation in solution.

(3) The constancy of the reaction during these inflections is due to the fact that the oxyhæmoglobin is in the precipitated condition. Oxyhæmoglobin forms a separate phase in a reacting system when it is in the precipitated condition, but not when it is in colloidal solution.

(4) Hæmoglobin is to be regarded as a polyvalent colloidal ampholyte capable of giving rise to ions of various valencies in solution. It is suggested that the oxygen combining power of hæmoglobin is determined by the ionic charge it carries.

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THE MAXIMUM WORK AND MECHANICAL EFFICIENCY OF HUMAN MUSCLES, AND THEIR MOST ECONOMICAL SPEED. BY A. V. HILL, F.R.S.

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This paper deals (i) with experiments by which the maximum work performed by human muscles in a single voluntary contraction may be determined, and (ii) with the various factors affecting the work done in, and the mechanical efficiency of, muscular movement in man.

Previous work on the isolated muscle has made it clear (1 & 2) that in an isometric twitch the force developed (T) is a measure of the mechanical energy liberated, and that T is related to H , the total energy set free, by certain comparatively simple relations when the temperature, strength of shock, and initial length are varied (2). In a twitch there is evidence (1, p. 151) that the theoretical maximum work of a muscle (i.e. the potential energy set free) is some constant fraction of Tl , l being the length of the muscle; and in the case of the frog's sartorius, this fraction was found (2) to be about $\frac{1}{6}$. The proportion existing between the mechanical energy available, and the quantity Tl , has been confirmed by Doi (5, p. 340), employing a "maximum work" device described by myself (3 & 4); Doi showed that W , the maximum work performed when a frog's sartorius is allowed to shorten, bears a constant ratio to T , the force developed when the muscle carries out an isometric contraction in response to the same shock, and under the same initial conditions. In actual practice however the maximum realisable work was much less than $Tl/6$, viz. at 15° about $\cdot 043 Tl$. The difference would seem to be due to the phenomenon described by Hartree and Hill (6), who found that when the length of a muscle is altered passively a considerable quantity of heat is evolved, which is greater the more sudden the change of length; this degradation of mechanical energy they ascribed to viscous resistance to a change of form, and it is obvious that any agency which degrades mechanical energy into heat when a muscle changes its form passively must work equally when the change of form is caused by the activity of the muscle itself. The response therefore of a frog's muscle to a single shock is so rapid that only about $\frac{1}{6}$ of the total

mechanical energy set free, at 15°C ., can be realised experimentally as work; the remaining $\frac{3}{4}$ is degraded into heat, owing to the viscous resistance of the muscle to a rapid change of form. If the twitch could be made to last longer the change of form by which the work is obtained would not need to be so rapid, and more work would be done, provided that the viscous resistance of the muscle to change of form were not increased at the same time. A fall of temperature increases the duration of a twitch, and Doi's results at 5° give an average value of $\cdot 051$ for W/Tl , which is some 20 p.c. greater than his average $\cdot 043$ for 15°C .; the reason why the difference is not greater is presumably that a fall of temperature has simultaneously increased the viscosity of the muscle. The contraction however can be, and in ordinary life is, increased in duration by another means, viz. by increasing the duration of the stimulus, and we should expect that the realisable maximum work would become a larger fraction of the theoretical maximum as the duration of the contraction is increased. This deduction has not yet been verified on isolated muscle, but the experiments described below show that it is true in the case of human muscle. In order to make the argument clearer, it is proposed to adopt the terms "realisable maximum work," and the symbol W , to mean the maximum work obtainable by any actual experimental means, from a contraction of any given duration; and the term "theoretical maximum work," and the symbol W_0 , to mean the mechanical potential energy set free, i.e. the maximum work which would be obtained were the resistance of the muscle to rapid change of form to be abolished, or in other words $W_0 = W +$ (the mechanical energy degraded in the rapid change of form).

In a single twitch there is evidence (1 & 9) that the mechanical potential energy set free, i.e. W_0 , is a large fraction, nearly 100 p.c. of the total initial heat-production. In the oxidative recovery process (10) about as much heat is liberated as in the combined initial processes of contraction and relaxation, a fact which has been confirmed more exactly by recent, hitherto unpublished, experiments. Thus the total energy liberated in, or as a result of, a twitch is equal approximately to $2W_0$, so that the theoretical efficiency is about 50 p.c. In a prolonged contraction the "initial" heat-production (1) is made up of two parts, one representing and equal to W_0 , the potential energy set free, the other proportional to t , the duration of the stimulus; thus in all cases we may write the total heat, $H = 2(W_0 + bt)$, where b is some constant. Thus the theoretical efficiency W_0/H has its maximum value of 50 p.c. when $t = 0$, and diminishes continually as t is increased. The actual efficiency W/H be-

haves in a different manner, and passes through a maximum value as t is increased; this we shall show below.

In order to obtain the maximum work from a contracting muscle it is necessary to oppose its contraction at every stage by a force which it is only just able to overcome. The use of a smaller opposing force wastes some of the mechanical energy, of a larger opposing force stops the contraction altogether. The "isotonic" system possesses both disadvantages; at the commencement of the contraction, work is wasted because the "load" is too small, at the end the shortening cannot continue because the "load" is too great. Apart from the use of complicated cams, or of complicated electromagnetic devices, the only practicable means of securing the right-at-all-stages load would appear to be to oppose the muscle to the inertia of a mass whose "reaction" (in the Newtonian sense) would always be equal to the force applied to it by the muscle. It is not convenient experimentally to oppose the contraction of a human muscle directly by the reaction of a suspended mass, the mass required being far too large (up to half a ton or more) and incapable of variation. It is necessary therefore to employ gearing, using a smaller mass but "gearing up" its reaction to the muscle. In the case of a frog's muscle the system suggested by myself⁽³⁾ and employed by Doi⁽⁵⁾ consisted of an arm balanced on knife-edges, and carrying two balanced masses, the "reaction" being "geared up" by allowing the muscle to pull at a point on the arm much closer to the knife-edges than the balanced masses. As a matter of fact, the design of this instrument was anticipated by A. Fick, who describes a similar device in a book⁽⁷⁾ which I had never previously seen, but which has been sent to me very kindly by Professor Meyerhof of Kiel. The rate at which the contraction takes place can be varied by varying the "gearing," in this case by varying the point of attachment of the muscle, or the distance of the balanced masses, in other words by varying the "equivalent mass" of the system. The "equivalent mass," which is measured by Mk^2/a^2 , Mk^2 being the moment of inertia of the system about the knife edges, and a the distance therefrom of the point of attachment of the muscle, is defined as that mass which, suspended freely and pulled directly by the muscle, would oppose the contraction by the same reaction as does the actual system considered; in other words the muscle, as regards the rate and force of its contraction, would be incapable of distinguishing between the actual system and its "equivalent mass." It is found that as the "equivalent mass" of the system is increased the work done in a twitch increases up to a certain maximum, and then decreases again;

this maximum is the "realisable maximum work" W . The reason why the maximum occurs is as follows; for a small equivalent mass the shortening of the muscle is too rapid, and much of its mechanical energy is dissipated in overcoming its viscous resistance to the rapid change of form; for too large an equivalent mass the shortening is too slow, and is not complete before relaxation has begun, so that some of the mechanical energy is never realised at all. Apart from the onset of relaxation there is no doubt that the work done would increase continually with the equivalent mass, until it finally attained asymptotically the theoretical maximum W_0 .

In the case of human muscles it is not practicable to use a maximum work device of the kind referred to above, as it would be inconvenient

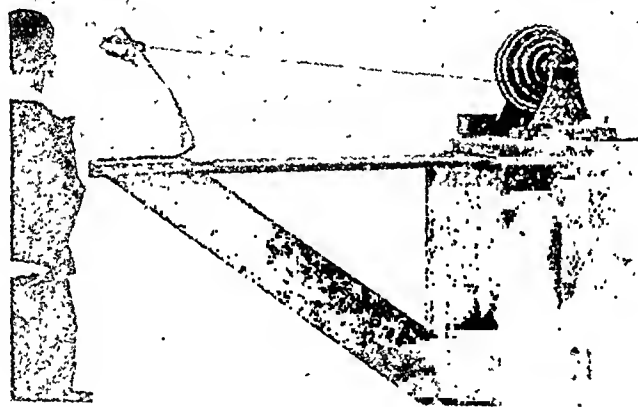


Fig. 1. Apparatus employed.

and unwieldy. It was decided therefore to employ a heavy fly-wheel, shown in Fig. 1, to provide the inertia against which the muscle has to work. A string is wound round one of the pulleys, and the subject of the experiment pulls the end of the string, employing only the biceps and brachialis anticus muscles as described below, and producing rotation of the fly-wheel. The speed of rotation is measured by a hand tachometer of the type D. 31 supplied by Messrs Moul & Co. of Westminster, and the work developed calculated, after preliminary calibration, from the deflection of the tachometer. Variation of the "equivalent mass" of the load, H , is obtained by winding the string round one or other of the different sized pulleys of the fly-wheel. The fly-wheel itself, with spindle, weighs 35 kilos., and was made from an iron casting. The shaft on

which it runs is of mild steel, and the wheel was accurately turned up between centres on its own shaft. The shaft runs in $\frac{3}{4}$ -inch ball-bearings, mounted in the cast-iron standards shown in the figure. It has altogether (counting the shaft itself) eight different sizes of pulley on which to wind the string. Each pulley is provided with a short steel peg, projecting some 4 mm., over which a loop at the end of the string is dropped before it is wound round the pulley. When the string unwinds the loop drops off the peg and leaves the fly-wheel free to revolve. The effects of friction are negligible during the time required to obtain a reading with the tachometer. After each reading the fly-wheel is stopped by pressing a block of wood against it. The kinetic energy in kilogram-metres corresponding to a given reading of the tachometer is obtained, and the instrument calibrated, as follows. A mass of about 16 kilograms is hung on a string, which is wound round one of the pulleys (preferably the smallest one) and the fly-wheel twisted till the mass has been raised through a measured height. It is then allowed to drop, the speed of the wheel so produced being measured by the tachometer. Allowance being made for the small amount of kinetic energy developed in the falling mass itself, it is then found experimentally that the work done by the mass is (as is necessary theoretically) proportional to the square of the angular velocity generated, and the constant c of this proportion enables W , the work done (in kilogram-metres), to be calculated from the reading r (in revs. per min.) of the tachometer by means of the formula, $W = cr^2$.

The "equivalent mass" of the fly-wheel is determined as follows. Suppose the moment of inertia about the shaft to be Mk^2 , and the string to be pulling with a force F on a pulley of radius a . If ω be the angular velocity of the wheel, $Mk^2 d\omega/dt = aF$, or $(Mk^2/a^2) (ad\omega/dt) = F$. But $ad\omega/dt$ is the linear acceleration of the edge of the wheel, so that the string (and the hand of the subject) are accelerating at the same rate as they would if pulling horizontally at a large mass Mk^2/a^2 hung upon a long string. Thus Mk^2/a^2 is the "equivalent mass" of the system, and so far as the muscle is concerned the contraction is precisely similar to one taking place against the inertia of a mass Mk^2/a^2 suspended freely. By making a sufficiently small, Mk^2/a^2 can be made sufficiently large. The equivalent masses of the system employed, using the eight different pulleys, are respectively in kilograms:

579, 308, 189, 66.4, 35.1, 21.8, 13.55, 11.3.

This is a wide enough variation for most purposes. The value of Mk^2 is obtained from the calibration described above, and the value of (a) from

the total length of string making two or three circumferences of the pulley. It is necessary, in making experiments of this type, to ensure that only certain definite muscles are used, that no appreciable kinetic energy is developed in the limb employed, and that the experiment is reasonably simple for an untrained person to undertake. For these reasons it was decided to employ the flexion of the arm, in such a position that the biceps and brachialis anticus muscles were the only muscles involved. The subject stands upright, with his (or her) arm stretched sideways horizontally, and resting on the flat support shown in the figure, with the side of the body pressed firmly against the support, and facing straight forward. It is necessary to provide boards to raise the subject to the height required, to enable the stretched horizontal arm to rest on the support. He then grips the handle attached to the string firmly in his hand, palm upwards, and the string is wound round the pulley by the observer until the arm is just fully extended. The length of the string is so adjusted that a full contraction of the arm leaves a few centimetres of the string still on the pulley, which falls off freely as the fly-wheel continues its revolution. In this way the full tale of work may be done by the contracting muscles. A warning is given and then, at a signal, the arm is flexed, as powerfully as possible, care being taken to maintain it in the lateral vertical plane, and so to avoid the use of other and more powerful muscles, and to ensure that the elbow never rises from the support. The movement should be treated as a piece of "drill," the body being held rigidly square, as at "attention," and no subsidiary movements of any kind allowed, either of the head, the trunk, or the legs. With a little practice, and by insisting on the subject following the routine, the movement is carried out with regularity and precision, successive readings on the same pulley agreeing accurately with one another. Professor J. S. B. Stopford, who has kindly advised me, agrees that when the movement is properly performed none of the work is done by muscles other than the biceps and the brachialis anticus. Without precautions, of course, serious errors may result, especially if the powerful pectoral muscles be employed.

The relation between the total work done in a contraction, and the equivalent mass.

The experiments were made on a variety of different individuals, and mean curves were constructed as shown in Fig. 2. In addition, one careful standard curve (Fig. 3) was made on the subject shown in Fig. 1, a powerful active man of 24 in good physical condition, by means of

repeated observations extending over three weeks; this curve will be used as the basis of calculation, as it is very accurate. The experiments shown in Fig. 5 were all made on the subject shown in Fig. 1. To determine the relation between the total work and the equivalent mass, the subject, after preliminary practice, makes a series of maximal pulls, starting usually on the smallest pulley (i.e. on the one corresponding to the greatest equivalent mass) and proceeding step by step to the largest (the least equivalent mass), then repeating the complete series in the reverse order. In this way two observations are obtained with each equivalent mass, and by taking the mean of these one eliminates the effects of fatigue (which are in any case small). Thus a series of readings is made relating the work done to the equivalent mass of the load, which when plotted give the curves shown in Fig. 2. Moreover, it is obvious that the work done against zero equivalent mass must be zero, so that the origin also lies upon the curve. In this way nine points are obtained on each curve. In Fig. 2 the curves represent (i) the mean of 22 men students in this laboratory, (ii) the mean of eight women students, (iii) the strongest student, and (iv) the mean of two children aged 7 and 6 years. The curves are easy to draw, the actual means for (i) and (ii) all lying accurately upon them. From these curves we see that, in all subjects, the greater the equivalent mass the greater the work done, the work increasing rapidly at first with equivalent mass and then more slowly, but continuing to increase up to an equivalent mass of over half a ton. The explanation, as pointed out above, is probably a simple one. The more rapidly a muscle shortens, the more the potential energy developed in it on stimulation is wasted in the passive and viscous processes associated with the change of form. Only by allowing a passively stretched muscle to shorten infinitely slowly can the full tale of potential energy be obtained from it, and what is true of the inactive muscle under a tension of external origin is almost certainly true of the active muscle under the tension of its own contraction. A quantitative confirmation of this view will be given below, in connection with the standard curve of Fig. 3.

The curves shown in Fig. 2 are instructive in themselves. The strongest man in 22 was able to do some 40 p.e. more work than the average of the remainder, and it is of interest that the three men giving the largest readings were all fast bowlers at cricket. Moreover, the average work of 22 normal young men is some 80 p.e. greater than that of eight normal young women. The curves shown in Fig. 2 are, in a general way, similar, the curves for women and children being like these for men but on a reduced scale: for a given equivalent mass the women did about $\frac{1}{2}$

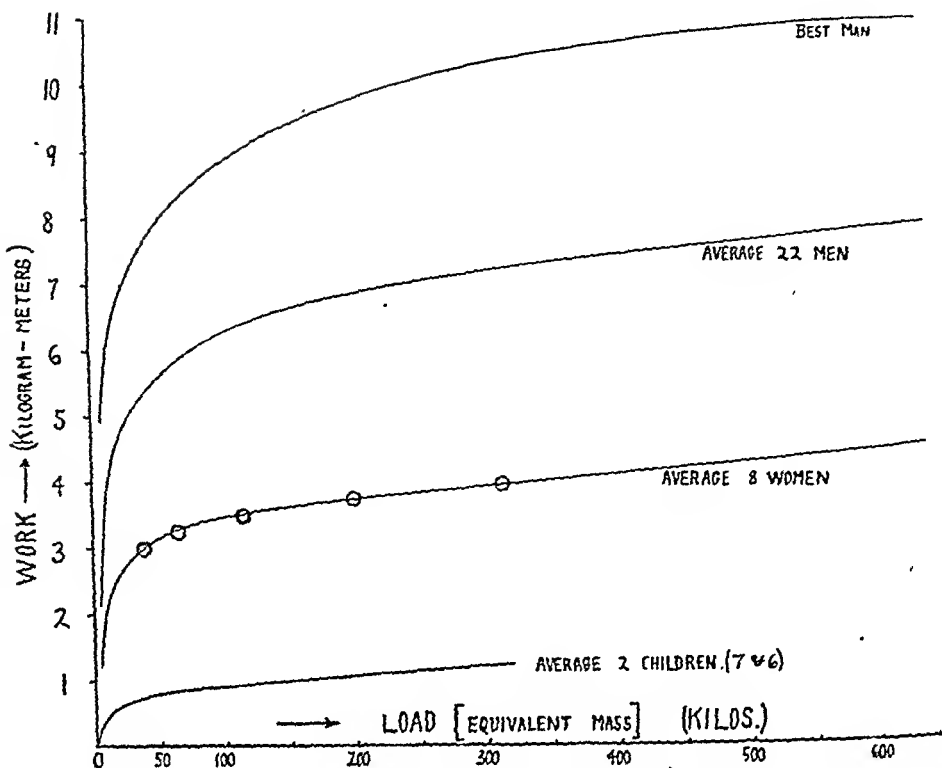


Fig. 2. Relation between maximum work W and equivalent mass M , in various individuals.

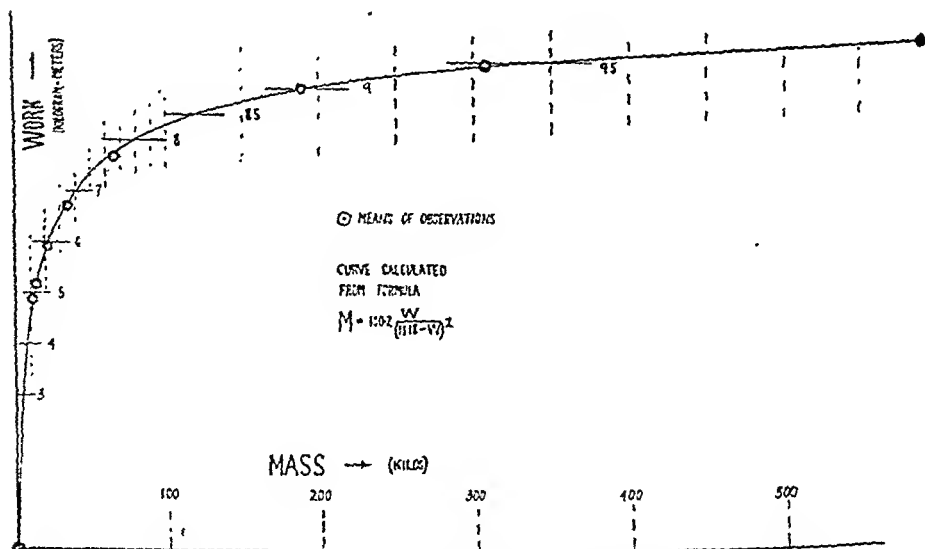


Fig. 3. "Standard" relation between maximum work W and equivalent mass M , obtained by repeating observation on one individual.

as much work as the men, and the children $\frac{1}{2}$ as much. The curves however are not exactly similar; if they were their final slopes would not, as in Fig. 2, be approximately the same. For a given equivalent mass the work done by the average man does not bear a constant ratio to that done by the average woman; the ratio decreases as the load is increased. The meaning of this, suggested by the theory of dimensions, is as follows. If we assume that the woman is able to impart to one kilogram exactly the same velocity as the man to two kilograms, then the men's and the women's curves become identical. We have merely changed the unit of mass, it being purely a matter of chance that the ratio is exactly two to one. Taking the men's curve, it is possible then to construct the women's curve as follows. With an equivalent mass of 50 kilos. a man can do just as many kilogram-metres of work, as a woman with 50 half-kilos. can do half-kilogram-metres of work; with 100 kilos. a man can do as many kilogram-metres as a woman with 100 half-kilos. can do half-kilogram-metres; and so on. Working with this rule the points shown with circles on the women's curve have been calculated from the men's curve. The agreement is perfect. Thus to a healthy normal individual, and for a given type of response, we may allot a "strength constant" λ , such that he can impart to any mass $M\lambda$ the same velocity as some standard individual can impart to the mass M . Expressed in terms of the average man of Fig. 2, the "strength constant" of the average woman, for this particular movement, is 0.5.

Another striking fact is the large amount of work done by the muscles. The "average man" on the smallest pulley does some 8 kilogram-metres of work. By the kindness of Dr G. E. Birkett of the Manchester Royal Infirmary I have been able to obtain an estimate of the mass of the muscles involved; the biceps and brachialis anticus together of a fairly muscular man weighed about 250 grams. Assuming this to be the mass of the muscles, in the case of the average man, each kilogram of muscle is able to do some 32 kilogram-metres of work in one contraction, an amount sufficient to raise its own weight through 32 metres. If the whole of this mechanical energy were degraded into heat the rise of temperature of the muscle would be about 0.076°C . Actually the rise of temperature of the muscle is much greater than this, partly because the oxidative recovery process gives another 0.076°C ., partly because the energy dissipated in maintaining the contraction is considerable. The time occupied in the pull against the greatest equivalent mass is some $2\frac{1}{2}$ secs., and if the heat-production of a man's muscle, exerting a maximal response at 37°C ., could be extrapolated from that

of a frog at lower temperatures (1, p. 138) the total rise of temperature of the human muscle should be about 2°C . This estimate is probably excessive, but the fact that the rise of temperature is large agrees with the subjective impression that considerable energy is expended by the muscles in these movements.

In a recent paper Hartree and A. V. Hill (8, p. 119) gave experiments, in which the maximum work of frog's muscles in a prolonged contraction was recorded. A pair of sartorius muscles weighing 0.33 gm. did 95 gm. cm. of work in response to 0.4 sec. tetanus at 21°C . With a greater equivalent mass, and a more prolonged contraction, the work might conceivably have become as great as 200 gm. cm., a quantity sufficient to raise the muscle through 6 metres. This is less than $\frac{1}{4}$ of the 32 metres attained by the human muscle.

It is possible, with certain simple assumptions as to the viscous resistance of the muscle to change of form, to calculate from first principles the shape of the curve in Fig. 3. The pressure required to drive a fluid through a capillary tube of given dimensions, is proportional to the coefficient of viscosity of the fluid and to the volume forced through per second. If we wish to increase the velocity with which the fluid flows through we must increase the pressure in the same ratio. Thus the work done in the transference of a given amount of fluid through the capillary, which is equal to the pressure multiplied by the volume, is proportional to the velocity with which the process is carried out. The change of form of a muscle involves the flow of fluid through the protoplasmic or colloidal network and the visible structures of the cells, and a given change of form is similar, in general character, to the flow of a given amount of fluid through a set of capillary tubes. Thus the mechanical energy degraded into heat should be proportional to the rate at which the given change of form is caused. Suppose a muscle, when undertaking a maximal contraction, to possess mechanical potential energy W_0 ; then if the shortening be against the reaction of an almost infinitely great mass, it will be very slow, and practically the whole of the mechanical energy will be realised as work; if however the mass providing the reaction be finite, the change of form will proceed at a finite rate, and a finite proportion of the mechanical energy will be degraded into heat; only the remainder, W , say, being realised as work. Thus $(W_0 - W)$ is equal to the energy dissipated in the shortening, which, from above, we should expect to be proportional to the velocity with which the shortening is carried out. Now it is shown experimentally below that the external force actually exerted by the arm in pulling a given mass may be taken as constant throughout the pull, varying however with the mass. Let P be the average force exerted on mass M ,

and l the length of the pull. Then $W = Pl$, and the time t occupied in the pull is given, according to the simple laws of mechanics, by the formula, $l = \frac{1}{2}Pt^2/M$. Now the energy degraded, being directly proportional to the average speed of the change, is inversely proportional to t , so that, $W_0 - W = k/t$ where k is some constant varying as the coefficient of viscosity. Substituting $\sqrt{2lM}/P$ for t , $W_0 - W = k\sqrt{P/2lM}$. Putting W/l for P from above, squaring and rearranging, we then find, $M = KW/(W_0 - W)^2$, where K is another constant equal to $k^2/2l^2$. Suppose that $K = 100.2$ and $W_0 = 11.18$. Then the equation becomes $M = 100.2W/(11.18 - W)^2$, from which the curve shown in Fig. 3 has been constructed. The actual mean observed points are shown with circles, and the agreement between calculated and observed is very good, so good as to provide strong evidence for the accuracy of the theory. We may assume therefore that we are correct in ascribing the shape of the curves of Figs. 2 and 3 to the viscous resistance of the muscle to a change of form, and in supposing that the function of a stimulated muscle is to develop potential energy, which is then transformed into work to a degree depending on purely physical factors.

In this equation the quantity W_0 has been taken as 11.18. W_0 is determinable within 1 p.c. from the observations, so that by plotting the latter and fitting a curve to them the potential energy developed can be measured. Thus in the subject employed the potential energy developed is 11.8 kilogram-metres. If this be equated to $Tl/6$, and if l be put equal to 0.15 metre, we find $T = 450$ kilos.—about half a ton. It is difficult to realise the enormous force developed by comparatively small muscles. The quantity K is proportional to the square of the coefficient of viscosity of the fluids in the muscle, and inversely proportional to the square of the muscle's length. In a given subject, K can change only as the result of a change in the coefficient of viscosity of the muscle fluids, and it would be interesting to see how far alterations of K can be associated with alterations of bodily condition, or with treatment such as massage. Other things being equal, the muscle wasting less of its energy in the rapid change of form will carry out its movements with greater power and speed.

Assuming that the force exerted on the wheel by the arm of the subject is constant throughout any given contraction, an assumption shown to be true with sufficient accuracy by the curves of Fig. 5, the time taken in the shortening can be calculated from the relations given above, viz. $W = Pl$ and $l = \frac{1}{2}t^2P/M$. These give $t = l\sqrt{2M/W}$. In the subject employed in the observations of Fig. 3, l was about 60 cm. Thus

culution¹) to be of uniform section, does not exceed $\frac{1}{6} \cdot 1500 \cdot 300^2$ ergs. This is equal, approximately to 0.23 kilogram-metres and is an extreme value, introducing in the worst case an error of not more than 5 or 6 p.c. in the observed result. In the case of the slower movements associated with the higher values of the equivalent mass, the kinetic energy developed in the subject's forearm is quite negligible. Had observations been made at still smaller values of the equivalent mass (say 5 kilos or less) the kinetic energy produced in the forearm would have become an appreciable fraction of the whole kinetic energy obtained, and a correction would have been necessary. Over the range considered however the kinetic energy produced in the fly-wheel may be regarded as the only external mechanical effect of the potential energy developed by the muscle.

When employing a very small equivalent mass (say one kilo. or less) the kinetic energy of the forearm becomes a large fraction of the whole mechanical energy set free. In the case of ordinary voluntary maximal muscular movements, carried out against the reactions of small masses, this loss of energy is to some degree avoided (as in throwing a cricket ball) by the use of a jerk, by means of which the kinetic energy of the moving limb is concentrated at the critical moment in the part immediately in contact with the object. Success in any form of sport or athletics involving rapid movement must depend upon the development of nervous and muscular co-ordination enabling such concentrations of kinetic energy to be produced inexpensively at the right time and place.

The relation between the work done and the time in a single contraction.

To determine this relation it was necessary to make an instantaneous and continuous record of the velocity of the fly-wheel throughout a contraction. The first method tried was to connect to the fly-wheel by a belt a small dynamo, and to record on a string galvanometer the current produced by the dynamo. Had a suitable instrument been available, this method would doubtless have proved satisfactory. In lack however of a good small dynamo another method was used. A circular ebonite ring mounted on a brass bush was furnished with eight brass pins driven into radial holes uniformly spaced in the ebonite, and making electrical contact with the bush. The pins were cut off flush with the circumference of the ring, the bush was then driven on to the spindle of the fly-wheel, which was mounted between centres and the ebonite ring with the brass

¹ The K.E. of a uniformly rotating arm of mass M , the end of which is moving with velocity v , is $\frac{1}{2} Mv^2$.

pins in it turned accurately in a lathe. A phosphor-bronze spring, with a sharp V-shaped contact, rubbed upon the circumference of the ebonite ring, and another similar spring upon the spindle of the wheel. Each time that the surface of one of the eight pins came in contact with the V-shaped portion of the first spring an electrical connection was established between the two springs, and by introducing a battery and an electromagnet into the circuit it was possible to record accurately on a drum the moment at which each pin passed under the V. The electromagnet was of the small light type made by the Harvard Apparatus Co., and was capable of carrying out well over 100 complete movements per second; this is more than twice the number required in any actual

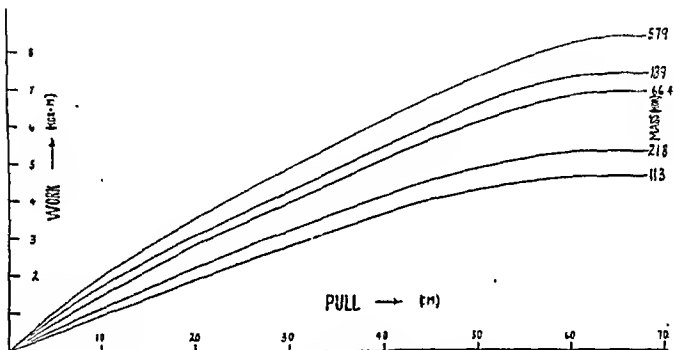


Fig. 5. Relation between work done and distance pulled in a single contraction; various loads.

experiment. The magnet was caused to write upon a rapidly moving drum revolving at a determined speed, so that the times between successive $\frac{1}{8}$'s of a complete revolution of the wheel could be read off on the record. The initial position of the V-contact (as a fraction of the interval between two pins) was noted, so that after measurement the relation between the angle through which the wheel had revolved and the time could be plotted. From the curve so obtained it is possible to calculate the angular velocity, and therefore the kinetic energy of the wheel, and to express it as a function either (a) of the time, or (b) of the distance through which the string pulled by the subject has moved. The former does not appear to be of much interest, and the results have been expressed, as shown in Fig. 5, in terms of the relation between work done

and distance pulled. It is seen that in all cases the work done increases, not exactly but more or less uniformly with the distance, showing that the force exerted on the wheel is approximately constant throughout a given pull, until the limit of the contraction is reached. The pull of an isolated muscle falls off rapidly as the muscle shortens, and the relative constancy of the force exerted by a man's arm is due to the increasing mechanical advantage of the lever system as the contraction proceeds. Since work is equal to the product of force and distance, the slope of the curves of Fig. 5 gives the external force exerted by the arm at any particular degree of contraction; the slope of any curve does decrease somewhat as the shortening proceeds, and this falling off is no doubt due to the more rapid movement at the end than at the beginning of the process, more of the total available force being required in the later stages to overcome the intrinsic resistance of the muscle itself to its more rapid change of form. The same fact is brought out by the different curves; at any given degree of shortening the slope of the curve for the greater mass is steeper than that for the smaller mass—showing that at every stage of contraction the force exerted is greater the less the rate of movement, and *vice versa*. The curves of Fig. 5 can be used for the calculation, by the simple rules of mechanics, of other factors in muscular contraction; it is unnecessary however to give such further calculation here.

It has been shown above that the slower the contraction the greater the work done. This does not mean however that the slower the contraction the more efficiently it is carried out, using the word "efficiency" as denoting mechanical efficiency, the ratio of work done to energy degraded in doing it. The more prolonged contraction necessarily involves a greater degradation of energy in the *physiological* processes necessary to maintain the contraction, and this factor rapidly neutralises the advantage of obtaining more work from the more prolonged contraction. The relation established, for maximal isometric contractions, between the total heat-production H and the duration of the stimulus t , may be written as shown above,

$$H = 2(W_0 + bt),$$

where b is some constant. It is clearly impossible to determine b by the direct thermoelectric method employed with the sartorius of the frog; it might however be possible to obtain an approximate estimate of it by experiments on the oxygen consumption of a man sustaining a maximal isometric effort. In the case of the experiments shown in Figs. 3 and 4, $W_0 = 11.18$ kilogram-metres; b is not known, but for the sake of cal-

calculation it has been assumed to be 5 in the following calculation. There is strong indirect evidence given below that this value is not far wrong; in any case the chief points in the argument are independent of the actual value of b , it being necessary to allot a number only for the sake of drawing an actual curve (Fig. 5).

The work done also increases with the duration of the stimulus, following the equation $W = W_0 - k/t$. Here $W_0 = 11.18$ and $k = 2.7$. The mechanical efficiency therefore is

$$E = W/H = (W_0 - k/t)/2(W_0 + bt)$$

One conclusion can be drawn at once from this experiment the efficiency E passes through a definite maximum value as the duration of the contraction is increased. The numerator ($W_0 - k/t$) increases at a decreasing rate, starting from zero when $t = k/W_0$ and finally attains a value W_0 . The denominator increases indefinitely at a constant rate from the finite value $2W_0$. Thus E starts at 0 and finishes at 0, as t increases, and must therefore pass through a maximum value. This maximum value of the efficiency corresponds to the most economical speed of working, the size of the pulley determining what we may call the "optimum gear ratio." Putting $W_0 = 11.18$, $k = 2.7$, $b = 5$, the mechanical efficiency E may be calculated and plotted, as in Fig. 6. It is seen that the efficiency starts from zero (at a time equal to that occupied in the flexion of the unloaded arm), rises rapidly to a maximum of about 26 p c., and then slowly falls again, as the duration of the contraction is increased. The actual height and position of this maximum depend upon the value of b , all the other constants being known. This maximum can be shown mathematically to occur at a time given by

$$t = k/W_0 [1 + \sqrt{1 + W_0^2/bk}]$$

and for different values of b these "optimum times," and the corresponding maximum efficiencies, are as shown below.

b	0	1	2	3	4	5	6	7	8	10	12
Optimum t	∞	1.91	1.43	1.22	1.10	1.02	.95	.90	.87	.81	.77
Maximum E	0.5	.374	.330	.303	.281	.263	.248	.234	.223	.204	.188

There is evidence from the work of Douglas⁽¹¹⁾ that the mechanical efficiency of human muscular movement may rise to 25 or 26 p c. Benedict and Cathcart⁽¹²⁾, in observations controlled by no-load experiments with a motor-driven ergometer, found that the average efficiency in all experiments was not far from 27 p c., but they state that

owing to extraneous muscular movements in the control experiments this value is probably too high. They obtained still higher values for the efficiency by taking as a "base-line" an experiment with a lower rate of work, but in view of the conclusions reached below as to the lower efficiency of a submaximal effort these values would seem to be unreliable, and we are probably justified in assuming that the maximum efficiency of human muscular movement lies round about 25 p.c. It is striking that Benedict and Cathcart expressly emphasise that the

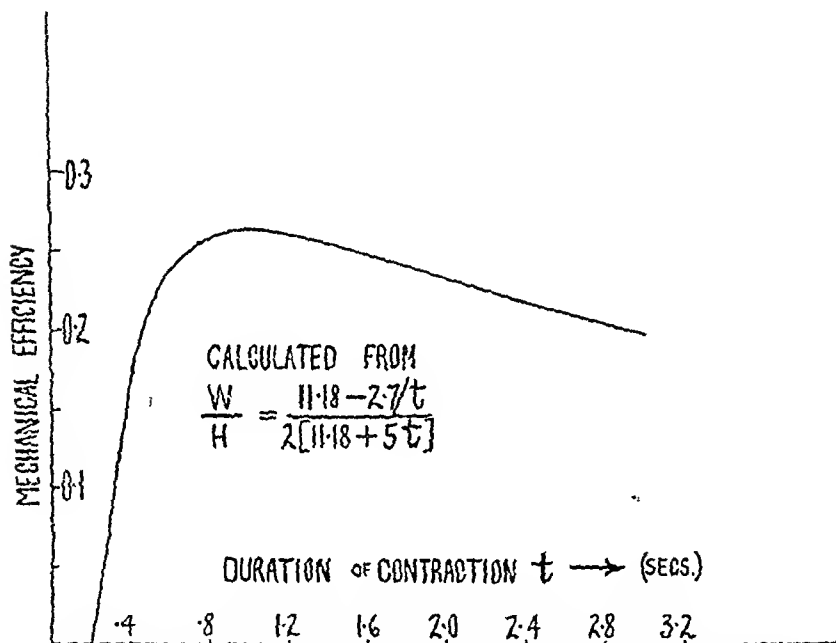


Fig. 6. Calculated relation between mechanical efficiency and duration of contraction.

efficiency decreases with a high rate of pedalling. This entirely agrees with the deduction given above, and with the curve of Fig. 6.

Assuming that 25 to 26 p.c. is the maximum mechanical efficiency attainable we are able, from the above table, to make an approximate estimate of the quantity b in the expression for the heat produced. This would seem to be about 5.5, in the movement and with the subject investigated here, in which case the curve of Fig. 6 is approximately the correct one. The time occupied in the most efficient contraction, *i.e.* the "optimum time," would be about 1 sec. Benedict and Cathcart found the maximum efficiency of a subject pedalling a bicycle ergometer to occur at about 70 revolutions per min., *i.e.* each contraction occupied

just under 1 sec. This is in striking agreement with the deduction given above, and confirms the general validity of the theory. Assuming the value of b to be about 5.5, the equation for the total energy set free in a maximal effort becomes approximately $22.36 (1 + \frac{1}{2}t)$. This depends upon the mass of the muscles involved, and upon their condition and training, but—for a given condition and degree of training—we may assume that the same relation holds for other muscles, in the form

$$H = 2W_0 (1 + \frac{1}{2}t).$$

Moreover the work done in a maximal effort, $W = 11.18 - 2.7/t$, also depends upon the mass of muscles and upon their condition and training, and again—for a given condition and degree of training—this may be written, for any muscle,

$$W = W_0 (1 - 0.24/t).$$

Thus the efficiency of any maximal muscular movement may be expressed approximately by the relation

$$E = W/H = (1 - 0.24/t)/(2 + t).$$

This relation between E and t is very nearly the same as that shown in Fig. 6.

The rapid rise and the slow fall of the curve relating E to t is of fundamental interest. It shows (i) that by *decreasing* comparatively slightly the time occupied in a muscular movement a serious loss of efficiency may be caused, but (ii) that a comparatively large *increase* in the time may cause only a small loss of efficiency. Moreover the maximum is shown to be a very "blunt" one; over a wide range of speeds the efficiency remains more or less constant. This is doubtless the reason why all observers working on the mechanical efficiency of the body have obtained values round about 20 p.c. to 26 p.c.: their subjects have instinctively worked within the wide limits giving something near the maximum efficiency.

Hitherto we have dealt entirely with maximal muscular efforts. A submaximal effort is probably nothing but a maximal effort of some of the muscle fibres, the inactive fibres having their form changed passively by the activity of their neighbours. Thus the potential energy set free, or the maximum theoretical work, is proportional to the fraction n of the fibres taking part in the contraction, and may be called nW_0 . The heat-production also, $2(W_0 + bt)$, is reduced in the same ratio, and becomes $2n(W_0 + bt)$. The energy wasted however in the change of form has not been altered by the submaximal nature of the effort, since

the change of form has been the same, and has (we assume) occupied the same time. We may write the energy wasted therefore as k/t , as before. The mechanical efficiency then becomes,

$$E = (nW_0 - k/t)/2n(W_0 + bt) = (W_0 - k/nt)/2(W_0 + bt).$$

This is always less than the quantity $(W_0 - k/t)/2(W_0 + bt)$, since n is less than 1, and for weak efforts may become very considerably less. Thus a submaximal effort is always less efficient than a maximal effort occupying the same time, and, in general, the weaker effort is the less efficient. Moreover, the formula for the "optimum time,"

$$t = k/W_0 [1 + \sqrt{1 + W_0^2/kb}]$$

clearly shows that if k is increased to k/n , as in the submaximal effort, the optimum time is increased. The highest efficiency of a submaximal effort is obtained in a slower contraction than that of a maximal effort.

These facts are of importance in relation to methods of observing the mechanical efficiency in man. In such observations, in order to eliminate the error in the heat-production introduced by the basal metabolism B , the work done W , and the heat-production H (calculated from respiratory measurements), have been measured at two different levels of the power exerted, the efficiency being calculated then from the formula, $E = (W_1 - W_2)/(H_1 - H_2)$. It has been assumed that in this way the constant "basal" heat-production B has been eliminated by subtraction. This might be correct. The assumption however has been made implicitly that the efficiency of the less powerful effort, viz. $E_2 = W_2/(H_2 - B)$, is the same as that of the more powerful effort, viz. $E_1 = W_1/(H_1 - B)$. Only if this assumption be made do both E_1 and E_2 become equal to $(W_1 - W_2)/(H_1 - H_2)$. Now we have shown that the efficiency of a weaker effort is necessarily less than that of a stronger effort, so that E_2 is less than E_1 . Hence the efficiency determined in this manner is liable to a serious error, and results obtained by it must be discarded. It can be shown moreover that efficiencies thus measured will be too high, and it is striking in Benedict and Cathcart's paper that the highest values (up to nearly 50 p.c.) were so determined. The most reliable method would appear to be another they adopted, viz. the use of a "base line" in which the ergometer is driven artificially, by a motor, at the same speed as in the actual working experiment.

All these facts have their importance in ordinary and industrial life. It is not contended that the purely muscular factor discussed here is

the only important, or the most important, of the many factors which determine the optimum conditions of work it is one factor however, and the simplest one, and a scientific study of it may at any rate help us to understand the optimum conditions and so to apply them in our ordinary life.

The results obtained here have a possible application to heart muscle. It is shown that the more slowly a muscle contracts the more of its potential energy W_0 is liberated as work W . In a heart, the beat corresponds to a single twitch of a skeletal muscle, and it would seem likely that no more energy is used up in a long beat than in a short one, hence if the theoretical maximum efficiency W_0/H be constant, the realisable efficiency W/H is greater in a slow beat than in a rapid one, and this may be one reason for the fact that athletic individuals tend to show a very slow beat. It should be possible to investigate the matter on the isolated heart, either by purely mechanical experiments on a dead heart or by observations on a surviving one.

My sincere thanks are due to Mr A. C. Downing of this laboratory, who not only constructed the special instruments employed, but was responsible for their detailed design, and assisted me continually in the observations recorded here.

SUMMARY.

(1) An instrument is described by means of which the maximum work of human muscles (biceps and brachialis anticus) can be determined. This instrument employs the inertial reaction of a fly-wheel to take up the pull of the muscle, the work done being calculated from the speed of revolution of the fly-wheel, as measured by a hand tachometer of standard pattern.

(2) Experiments are given relating the maximal work W in a maximal contraction to the "equivalent mass" M of the load. As M increases W increases also, at first rapidly and then more slowly, tending to reach a definite final value W_0 , equal to the potential energy set free. It is shown experimentally that M and W are connected by the relation $M = KW/(W_0 - W)^2$ where K is a constant.

(3) In a maximal effort the duration of the shortening may be changed by changing the load. The greater the duration t the greater will be the work done W . It is shown experimentally that W and t are connected by the relation $W = W_0 - k/t$, where k is a constant. In this equation t is not less than the time occupied in a maximal contraction without load.

(4) These facts and relations can be deduced quantitatively from the hypothesis that a muscle, when stimulated, produces potential energy W_0 , which in any actual contraction is employed partly in doing external work W and partly in overcoming the viscous resistance of the muscle to its change of form. The energy dissipated ($W_0 - W$) in overcoming viscous resistance to a given change of form should be proportional to the speed ($1/t$) with which the change is carried out, and to the coefficient of viscosity (k) of the muscle fluids. This hypothesis leads to the preceding mathematical relations, and is in keeping with what is known as to the "thermo-elastic" properties of muscle.

(5) An instrument is described enabling a continuous record to be deduced of the relation between the work done and the distance pulled, in the course of a single contraction. Curves are given showing this relation for a variety of loads.

(6) The mechanical efficiency of human voluntary movement is discussed. It is shown that the total energy H set free in a maximal contraction of duration t is given approximately by the relation $H = 2W_0(1 + bt)$, where W_0 has the same meaning as before, and b has a value about equal to $\frac{1}{2}$. From this the mechanical efficiency W/H may be calculated as a function of the duration of the contraction, and it is shown that there is a certain optimum speed of movement below which the efficiency falls slowly, and above which it falls rapidly.

(7) The mechanical efficiency of a submaximal effort is always less than that of a maximal effort occupying the same time, and in general the stronger effort is the more efficient. Moreover the stronger effort has the greater optimum speed.

(8) The bearing of these conclusions upon results obtained by the use of a bicycle ergometer, as well as upon human muscular movement in general, is discussed.

The expenses of this research have been borne in part by a grant from the Government Grants Committee of the Royal Society.

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ADDENDUM.

It may be objected that the diminution of work with increased rate of shortening is due, at least in part, to the fact that with a small equivalent mass, an appreciable amount of the shortening has occurred before the full force of the contraction has had time to develop. This is not so. Experiments, which will be described in a later paper, have been made on the rate of development of an isometric contraction in the biceps and brachialis anticus muscles of the subject of the observations described above. These experiments have shown that the development of the contraction is so rapid that, even in an extreme case, not more than 0.5 p.c. of the total work is lost by this cause, while in the more prolonged contractions, the loss is entirely negligible. The loss is due to the actual process of shortening, not to the fact that the shortening occurs before the maximum force has been developed.

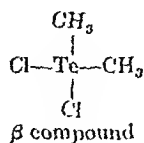
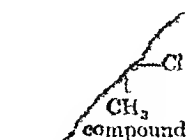
THE ACTION OF DIMETHYLTELLURIUM DIHALOIDS.

BY THE LATE DOUGLAS V. COW AND W. E. DIXON.

(From the Pharmacological Laboratory, Cambridge.)

THE internal administration of tellurium compounds has long been known to produce a garlic-like odour in the breath and from the skin; at one time this was commonly observed in those taking bismuth salts in which tellurium was apt to be an impurity. Reiser(1) found that .0005 mgrm. TeO_2 taken by the mouth produced this odour in $1\frac{1}{4}$ hours and that it lasted some thirty hours; after taking 15 mgrms. the odour was still obvious after 237 days. Hofmeister(2) and his pupils proved that this odour was due to $\text{Te}(\text{CH}_3)_2$ and that in whatever form tellurium was administered the methyl compound could be detected in all parts of the body: dogs, rabbits, frogs, worms and crustacea can all bring about this change. Mead and Gies(3) showed that non-toxic doses of tellurium in various forms did not materially alter metabolism in dogs; large doses caused vomiting and later sleepiness, and toxic doses, after a period of restlessness, caused gradual paralysis and death from respiratory failure. Tellurium compounds (tellurates) may be regarded then as exerting little toxic action in higher animals, except when administered in very large doses, although certain bacteria are highly sensitive to them(4).

The present experiments were made with two isomeric dimethyl-telluronium dichlorides $(\text{CH}_3)_2\text{TeCl}_2$ which were prepared in the Cambridge Chemical Laboratory by Dr R. H. Vernon(5). Both are quite stable and soluble in water, both the halogens are ionisable and can be precipitated quantitatively by silver nitrate. Dr Vernon suggests for these two isomerides the following formulæ which may be expressed in



the simple compounds of quadrivalent tellurium two corresponding with one structural formula, it is clear that with a new form of isomerism.

Apart from the action of tellurium we believed that an investigation of these compounds might afford some information on the relations between molecular structure and physiological action. The immediate action of these haloids, that is within a few hours shows no likeness to what may be termed a tellurium action: they each cause a specific and different type of effect on tissues. It is only when they are broken down in the body and excreted as $\text{Te}(\text{CH}_3)_2$ that what is commonly regarded as a tellurium action comes into play. This change, however, begins almost immediately and is probably complete in a few hours, since the characteristic odour then becomes faint.

Our experiments were made upon cats, dogs and rabbits, and the effects are in broad outline the same in all animals. The animals were anaesthetised with CCl_4 and urethane unless otherwise stated.

Immediate effect of intravenous injection. The injection of 5 mgrms of the β compound into the circulation of the cat or dog causes an immediate rise in the blood pressure with increased frequency and depth of respiration. This effect lasts 20 to 30 seconds and the blood pressure rapidly falls to normal. This is followed by a secondary rise which is usually more profound and always much more prolonged than the first effect (Fig. 1). When the whole action has passed off further injections may be made with similar results, but after each successive injection the effects become less pronounced: the initial stimulation of respiration and rise of blood pressure become shorter but the secondary rise in blood pressure remains constant provided some three or four minutes are allowed to elapse between each injection.

The injection of 5 mgrms of the α compound produces an entirely different type of effect: in the cat the heart immediately ceases to beat and the blood pressure falls to zero. This does not usually last longer than 20-30 seconds, then the heart suddenly begins to beat again and the blood pressure bounds up to a height above that before injection (Fig. 2). The effect is very similar to that obtained by injecting a concentrated solution of potassium chloride, and like the potassium effect it can be produced an indefinite number of times, unlike the β haloid the action of the α does not diminish with successive doses. Larger injections of the α haloid stop the heart beat for a longer period and if sufficiently large cause death. The heart, however, does not appear to be permanently injured as it is not difficult to reestablish its activity with massage even after standstill for some minutes. Smaller doses of α induce a fall of blood pressure lasting a minute or two without cessation of the heart beat again simulating a potassium effect. Respiration is little influenced

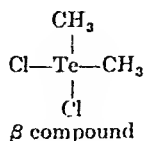
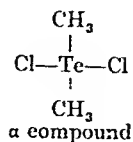
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These simple compounds of quadrivalent tellurium two corresponding with one structural formula, it is clear that with a new form of isomerism.

by the α compound: the immediate effect is no more than would be accounted for by the fall of blood-pressure.

The behaviour of the rabbit to the β compound is a little different from the cat and dog: only the secondary rise of blood-pressure occurs and in place of the first effect either no change is observed or a small and transient fall (Fig. 3).

Explanation of the vascular and respiratory effects. The primary rise in blood-pressure associated with the increased respiration which follows the injection of the β haloid suggests that the effect is central, due to stimulation of the medulla. If the spinal cord of an animal is severed below the medulla the primary rise of blood-pressure is almost or entirely deleted. Or if the central nervous system of an animal is completely paralysed by nicotine the β fails to produce any initial rise of pressure. This augmented pressure is mainly due to vaso-constriction and such constriction is easily shown by plethysmographic experiments in which a limb or loop of intestine is placed in an oncometer. The volume of the organ shrinks as the blood-pressure rises (Fig. 6). The vaso-constriction is certainly not peripheral for the reasons already given and because perfusion experiments on isolated organs show that the β compound never constricts vessels, that is diminishes the outflow from the vein, but rather tends to increase it.

The secondary rise in blood-pressure commences from 30-60 seconds after injection. It occurs in decerebrate animals and in animals in which the whole central nervous system has been paralysed by apocodeine or nicotine. This effect also is mainly due to vaso-constriction of peripheral origin but not due, for reasons already mentioned, to the direct action of the drug. This suggests that the β haloid may act on the suprarenal glands and that the rise of blood-pressure may be due to the liberation of adrenaline. If the suprarenal glands of an animal are ligatured and excised, the injection of β causes no secondary rise of blood-pressure (Fig. 4). Section of the two splanchnic nerves in the cat does not materially influence this secondary pressor action of the drug, although it usually cuts out almost completely the primary pressor action by separating the medulla from the splanchnic area. The β haloid does not excite any part of the nervous system directly, except the medulla (Fig. 5). For this reason we believe that it excites the suprarenal gland cells to activity directly and quite independently of the nerve-supply and suggest that it may be a useful substance with which to stimulate the gland for experimental purposes or to determine whether or no the gland is functioning. After repeated doses in quick succession the gland becomes

exhausted so that further injections produce little or no secondary pressor effect unless a rest is given when further injections again produce an

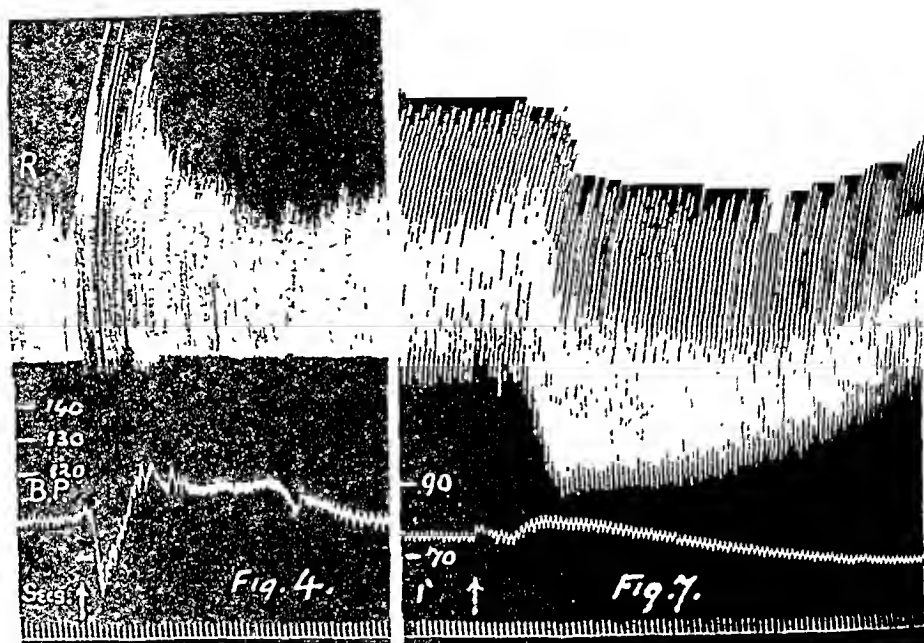


Fig. 4. Cat. Urethane. Splanchnics cut and both suprarenals ligatured off from the circulation. Injection 10 mgrms. β .

Fig. 7 Cat. Urethane. Cardiometer. B.-P. Shows an injection of 2 mgrms. β . The animal had already received 20 mgrms. β .

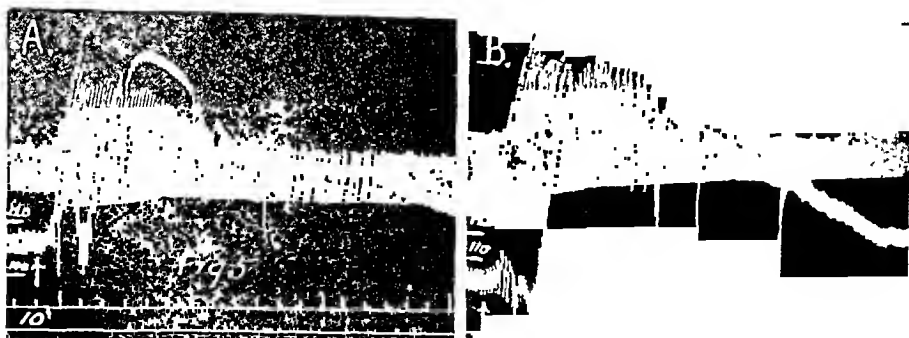


Fig. 5. Cat Urethane. B follows on A, but before B was taken the splanchnics were cut. Injection in each case 5 mgrms. β .

adrenaline action. We have endeavoured to verify this explanation by anaesthetising a cat slowly and quietly without undue excitement; one

suprarenal gland was then excised. The cat then received six intravenous injections of the β haloid, 60 mgrms. in all. Soon after the last injection the animal was killed and the second suprarenal gland excised. The two glands were weighed and made up into standard extracts with Ringer's solution and the activity of the extracts compared by testing their effects on isolated organs, rabbit's intestine and uterus, enucleated frog's eyes and on blood-pressure. Both extracts contained adrenaline however, although the larger amount was present in the extract of the gland first extirpated, but the difference was insignificant. This unexpected fact shows, if our explanation is valid, how rapidly the gland replaces the adrenaline it has lost.

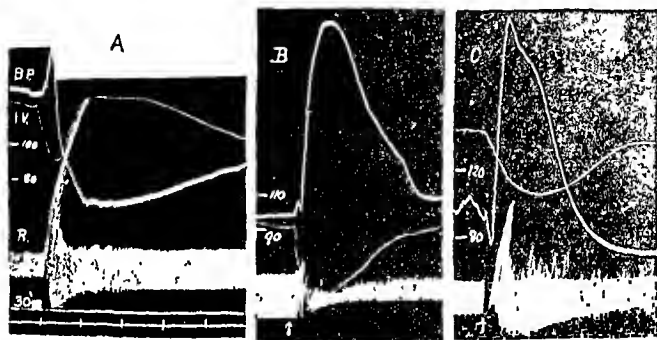


Fig. 6 Cat. Urethane. The intervals between A and B and B and C are four minutes. In A the fifth injection of β is shown (5 mgrms.). In B 5 mgrms. β injected. In C 5 mgrms. nicotine injected.

The depressor effect after repeated injections of β is shown in Fig. 6 A. This is apparently due to medullary depression which invariably follows profound medullary stimulation, but which after injections of β is generally masked by the pressor effect of adrenaline. The depressor effect is caused by vaso-dilatation. After a short rest of three or four minutes injections of the β haloid again cause a pressor effect from the liberation of adrenaline (Fig. 6 B); and there seems to be no limit to the amount of adrenaline which the suprarenal gland can manufacture provided a little time is given. An injection of nicotine is also shown in this figure to demonstrate the comparative effect between it and adrenaline (β) (Fig. 6 C). The respiratory curves in B and C are typical of

adrenaline and nicotine respectively, and the hump on the blood-pressure curve in *C* is also typical.

The depressor action of the α compound presents many similarities to that of potash compounds. If the heart of an animal is exposed when the haloid is injected, a few peristaltic-like waves can be seen to pass over the heart which then stops beating and becomes quiescent. Soon, however, the ventricles pass into a state of most intense fibrillation: if the dose has been too large this condition may not be observed unless the heart is gently massaged. The fibrillation generally lasts from two to five minutes and then, especially if aided by massage, passes off and the heart recovers completely. This effect is uninfluenced by the injection of atropine or calcium, and is caused by a direct action on the coordinating mechanism. The contractility of the ventricles returns a considerable time before auriculo-ventricular conductivity is complete.

Heart. From these effects on blood-pressure it is obvious that the β haloid should exert an adrenaline action on the heart in the second phase. This effect can be shown by recording the output of blood from the heart as in the cardiometer record in Fig. 7, p. 46. In this experiment the animal had previously received a large dose of urethane so as to obliterate the primary effect by paralysing the medulla: the action on blood-pressure is small but the effect on the heart is typical of that of adrenaline. The output of blood is increased and the heart empties itself more completely at each beat. This effect being the result of an action on the suprarenal glands is not produced in experiments on the isolated heart.

The α compound acts on the contractile tissue of the heart directly and causes standstill. If 5 mgrm. of α be injected into a lymph sac of a decerebrate frog arranged for recording the heart, the beat is first slowed in diastole, the height of contractions are slightly increased and death occurs in diastole a little after one hour: conduction is not altered. A like dose of the β compound injected under similar conditions first alters conduction; the A-V interval is increased, then the heart becomes irregular and later the ventricle contracts without reference to the auricles. Death occurs in diastole.

The introduction of 0.5 mgrm. of α into the coronary circulation of a surviving isolated rabbit's heart causes immediate ventricular standstill. The heart cannot be induced to beat again by the administration of calcium, atropine or adrenaline. Recovery may occur in time, but after the injection of 2 or 3 mgrms. the heart is killed, though the auricles continue to beat for some minutes.

The β compound produces a different type of effect. When the isolated

rabbit's heart is perfused with 1 in 50,000 β haloid it first beats a little more vigorously, after an hour, though still beating regularly, the tonus has risen considerably and in about two hours the heart dies in systole. Larger concentrations produce this effect in a shorter time. An analysis of the curves shows that in the later stages of poisoning heart-block is obtained just as in the frog, followed by complete auriculo ventricular arrhythmia. Sometimes the right ventricle contracts alone and sometimes with the left ventricle. Death always occurs in systole. Hearts poisoned in this way respond in a normal manner to pilocarpine and atropine, but calcium, physostigmine and adrenaline exert little or no action.

Action on some surviving organs. The α haloids cause plain muscle throughout the body to contract; this effect is definite though never decided. The β compounds have no peripheral action on plain muscle of note and any slight effect there may be is never towards contraction. The following experiments from a number will exemplify this. The action on isolated blood vessels was determined by perfusing the surviving hind limbs and intestines of cats and rabbits, and measuring the outflow from the veins. The following is a typical experiment.

Cat. Cannula inserted into aorta and inferior vena cava just before bifurcation. Perfusion at body temperature with defibrinated blood from the same cat diluted with an equal quantity of Ringer's solution. Experiment performed in a warm oven at 80 mm. mercury pressure. The figures represent the times in seconds for the collection of 10 c.c. from the vein.

Drug given	
30, 30, 28, 30,	α 32, 32, 25, 26, 25
36, 34, 34, 34,	β 32, 30, 32, 33

In each case 5 mgrms. of drug were injected into the tube connected with the artery.

The effect is trifling, but in every instance the α produces an initial constriction followed by dilatation.

On the uterus of the rat, guinea pig or rabbit this effect of α haloid is seen in a more decided form. The following is a typical experiment.

One horn of uterus of guinea pig suspended in 100 c.c. of oxygenated Ringer's solution at 38° C. The uterus is fixed below and attached to a lever suitably weighted so as to record on a smoked surface. The addition of 10 mgrms. 1 p.c. α is without effect. The addition of 10 mgrms. 1 p.c. β causes maximal contraction.

The same type of effects can be seen on the isolated intestine. These experiments were performed with both longitudinal and circular strips of rabbit's intestine and under conditions identical with those on the uterus. The addition of 5 mgrms. α slightly increased the intestinal tone and decidedly increased the contractions of the automatic movements. The β produced no effect.

the same electrical charge. The physiological action cannot therefore be due to the elements of the positive ion, nor yet to its electrons, since these are the same in both compounds, but to some other factor, that is the strained condition when the haloids are arranged in the *cis* position. Here then we have a clear case of a compound in which neither the elements composing the compound nor their crude arrangement in the molecule determine the physiological action. The highly specific action of the β compound must be regarded as due to some energy factor which holds the constituents of the molecule in an abnormal and strained position.

CONCLUSIONS.

1. Two tellurium dimethyl dihaloids are described, having the same structural formula but entirely different actions, though both are excreted in the breath as methyl telluride.

2. The α haloid arranged in the *trans* position is relatively inactive.

3. The β haloid arranged in the *cis* position acts as a powerful medullary stimulant and specifically excites the suprarenal glands. Large doses paralyse nerve structures in the following order: (a) sympathetic ganglion cells, (b) other autonomic ganglion cells, (c) medulla and motor nerve endings.

4. The suprarenal gland can manufacture almost unlimited amounts of adrenaline.

5. The physiological activity of the β haloid is due to intramolecular strain.

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THE REACTION OF RESTING AND ACTIVE MUSCLE.

By A. D. RITCHIE.

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From the heat production and lactic acid formation of muscles during activity it is possible to calculate how much lactic acid is concerned in a single muscle twitch. Hartree and Hill (1921) find that at temperatures between 10° and 15° C. a frog's muscle liberates 0.004 calorie per gram in one twitch. Peters (1913) showed that the heat liberated during activity or in rigor is proportional to the lactic acid formed, and from his experiments the ratio 1 grm. of lactic acid to about 450 calories can be calculated. Later work of Meyerhof (1920) with improved methods suggests 400 calories per gram as being more nearly correct. Using Meyerhof's figures it appears that the acid formed during a twitch is equivalent to a concentration of $1/9000$ normal. A completely dissociated acid of this strength would have, in pure water, a pH about 4. Even in rigor a muscle does not become so acid as this. Pechstein (1914) found the juice of muscles stimulated to fatigue to be almost neutral; pH 6.8 to 7.0. Even more striking is the evidence of CO_2 production. Any marked increases in acidity of a muscle will liberate CO_2 from bicarbonates, but Fletcher (1898) showed that moderate activity in air or nitrogen caused no increase in CO_2 output. Therefore the only conclusion possible is that the lactic acid concerned in the contractile process is not present in the free state even inside the cell, except momentarily.

The electric response, as Mines (1913) suggested, may be the manifestation of a sudden outburst of hydrogen ions and their migration from their place of origin to the contractile elements of the muscle fibre. The electric response begins a little before the mechanical response and is finished before the full tension of the muscle is developed. The muscle relaxes, as argued by Hartree and Hill (1921), and Meyerhof also, whom they quote, because the lactic acid is subsequently neutralised by the buffer substances in the plasma. The relaxed state, before oxidative recovery, differs from the resting state only in the presence of lactate ions in solution, in place of the negative ions of some weaker

acid, the ionisation of which has been suppressed. During the whole cycle of changes, on this theory, the hydrogen ions are fixed and are not free to wander out of the cell. Their only movement is towards the contractile structure, after stimulation until their fixation there. This fixation is marked by the development of the mechanical response and the dying away of the electric change. Hence the electric change should be the one and only manifestation of their activity, on Mines' theory. If Mines' theory is incorrect there is probably no observable effect due to free hydrogen ions.

The conditions under which hydrogen ions might be free and able to escape from the cell are those of fatigue or rigor when relaxation is imperfect or absent, that is to say, when the buffering action of the plasma is insufficient. Under these conditions it is well known that the muscle does become more acid and lactic acid and CO_2 can diffuse out. During oxidation recovery too, CO_2 must be liberated and there may be some small increase in acidity. However, quite apart from theory, the work of Fletcher on CO_2 production is very strong evidence against any changes in acidity during normal activity and in the absence of the oxidative recovery process. The electrometric experiments to be described are completely in accordance with this view and provide fairly direct evidence that no acid is liberated in the active muscle fibre in such a way as to be able to escape or to make any measurable change in its reaction. Changes in acidity are to be associated solely with impaired working of the muscular mechanism, such as fatigue or rigor.

The method of experiment used was to measure the difference of electrical potential between a resting and an active muscle by means of manganese dioxide electrodes. These electrodes are sensitive to hydrogen ion concentration, at least in a qualitative way. The electrodes used consisted of pointed platinum wires which were coated with electrolytically deposited MnO_2 . They were coated afresh before each experiment. A sciatic-gastrocnemius preparation was dissected out from one side of a frog. The muscle was arranged so that it was kept taut and rigidly held to avoid movement on stimulation. The preparation was set up in a moist chamber, the nerve hanging in air between the stimulating electrodes and the muscle to insulate the electrodes as well as possible. The other gastrocnemius muscle without its nerve was hung in contact with the first. The points of the electrodes were inserted into the muscles to the depth of a millimetre or two and connected up to a potentiometer. Any E.M.F. between them initially was balanced up and then one of the muscles stimulated through its nerve.

In some experiments a very sensitive galvanometer was used which recorded changes of less than $1/100$ millivolt. Afterwards, a less sensitive one was used and readings made to 1 millivolt. Various sources of error had to be eliminated. (1) It was found necessary to have the stimulating electrodes well insulated from the muscle. (2) The electrodes were very sensitive to movements of the fluid or tissue in contact with them. With electrodes on the surface of the muscles quite small movements of the muscles caused changes of E.M.F. (3) Even when undisturbed and in pure salt solution the electrodes showed perpetual irregular changes of potential. In contact with muscles these changes were even more marked. The muscle appears to produce some permanent change in the electrodes and to have some solvent action on the MnO_2 coating. (4) The electrodes were slow in responding to changes in the solution in contact with them and equilibrium might take several minutes to establish. Owing to (3) and (4) changes of a tenth of a millivolt or less could not be followed satisfactorily. (5) A rapid change of about one millivolt was always observed on stimulation and was clearly due to the electric response. It was obtained equally well with plain platinum or silver electrodes. If both electrodes were placed in the same muscle an analogous diphasic response was obtained. This change was always a small one due apparently to the long period of the galvanometer and the sluggish nature of the electrodes. Apart from this last change no alteration in the E.M.F. between the muscles could be observed as the result of stimulating one of them with 100 maximal induction shocks of a few seconds' tetanus. That is to say, the change, if any, was probably less than $1/100$ millivolt and certainly less than 1 millivolt. When a tetanising current was switched on and off there was no corresponding steady change of E.M.F.

The electrodes in phosphate mixtures of pH 6 and 7 respectively gave an E.M.F. of 0.07 to 0.08 volt, so that a change of 0.01 in the pH could have been detected. Under conditions which should, according to theory, have made the muscle acid, increase in acidity could certainly be detected by these electrodes. If the muscle was stimulated to fatigue, a slow rise in acidity was observed. By placing one electrode in salt solution into which the muscle containing the other dipped, it was possible to observe a large increase in acidity in chloroform rigor. The acidity rose to a maximum and then fell off, presumably owing to evolution of CO_2 . Injury production of lactic acid was observed by this arrangement. On first inserting the electrode the reaction was more acid than it subsequently became.

It is hoped to make absolute measurements of the reaction of muscle under various conditions by adjusting the reaction of the salt solution until there is no E.M.F. between it and the muscle. No very satisfactory results have been obtained so far because of the alteration in the electrodes that contact with the muscle brings about. But it would appear as the result of a few preliminary observations that resting muscle is more alkaline than pH 7.5, probably between 7.6 and 7.8. In chloroform rigor it becomes distinctly acid.

There seems no doubt that quite small changes in the reaction of muscles can be detected by this method, and that the negative result obtained is genuine and not merely instrumental. A considerable number of experiments have been made, and in none, once the sources of error were recognised, has there been any suggestion of a change of reaction in contraction or relaxation of a muscle. That the result does not contradict but supports the generally accepted view of the function of lactic acid in the contractile process must perhaps be emphasised again. The lactic acid undoubtedly alters the equilibrium of acids and bases in the cell and a chemical reaction of that sort probably plays a part in the contractile mechanism. But such a change need not cause more than a minimal alteration of hydrogen ion concentration if the cell is a well buffered system. The muscle cell, particularly that of red muscle, will contain all the buffer substances present in blood. It is only necessary to assume that the muscle plasma is a slightly alkaline medium composed of salts of weak acids with alkalis, an assumption supported by a certain amount of independent evidence. As the experiments here described contradict the results obtained by some previous workers, a criticism of their methods is necessary. Roaf (1913, 1914) attempted to measure the time relations of acid production. His method was to adjust two calomel electrodes on the surface of a frog's sartorius muscle preparation stimulated directly until no galvanometer deflection was obtained on stimulation. He then replaced one of the calomel elements by an MnO_2 electrode and assumed that any galvanometer deflection obtained then would not be due to the electric response but to a change in acidity. As, however, his calomel electrodes were non-polarisable and the MnO_2 electrode polarisable, it by no means follows that because similar electrodes were unaffected by the electrical change, dissimilar ones would be equally unaffected. The change he recorded was roughly contemporary with the mechanical response and might easily have been due to polarisation of the MnO_2 electrode due to the potential gradient set up by the electric change, an effect that would take slightly longer

to increase and decay than the electric change itself. The fact that the electrodes were in such a position that two non-polarisable ones were unaffected by the electric response would explain the fact that the change observed was monophasic. Another possible explanation of Roaf's results is that there was movement of the muscles under the electrodes. Lastly it is not certain that there was no spread of current from the stimulating electrodes.

The experiments of Galeotti (1906) and Porcelli-Titone (1914) who used hydrogen electrodes on muscles are equally open to criticism. The former states that resting reptilian heart has a pH 10, contracted pH 9, and killed pH 7. Porcelli-Titone found pH 7.8 for resting skeletal muscle and 7.2 for muscle stimulated to fatigue. These values are probable enough in themselves, but he found a pH between 7.0 and 7.4 under various conditions of moderate activity such as 200 single twitches. In these experiments the muscles were apparently in air. The hydrogen electrode consisted of a small glass chamber with an open end applied to the surface of the muscle. It contained a strip of platinum ending flush with the opening, and hydrogen could be passed continually through the vessel. It seems doubtful whether the tip of the electrode in contact with the muscle would ever be saturated with hydrogen, or whether errors due to movement of the muscle in contact with the electrodes were eliminated.

SUMMARY.

Electrometric observations indicate that there is no appreciable change in the hydrogen ion concentration of frog's muscle during moderate activity. This result contradicts certain previous experiments, which are criticised, but is in accordance with theoretical expectations.

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THE RELATIVE EXCRETION OF UREA AND SOME OTHER CONSTITUENTS OF THE URINE. By E. B. MAYRS.

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THE rate of excretion of some of the constituents of the urine has been the subject of frequent examination, and a contrast has been drawn between such substances as the chlorides and the sulphates or urea, the former as representative of the "threshold," the latter of the "no-threshold" substances (1). No such data appear available for the different members of the "no-threshold" group, and as a general rule it seems to be tacitly assumed that they are equally readily eliminated by the kidney; that is, that the urea and sulphate of the urine bear to each other the same proportion as the urea and sulphate of the plasma. This is the natural assumption as long as these are regarded as waste products simply, for, when it loses one of its constituents, the plasma must come into very intimate contact with the renal cells, and these may be expected to deal equally efficiently with any other useless constituent.

To determine this point I have performed a series of experiments on the excretion of the waste products of metabolism with which the kidney has ordinarily to deal.

The first tests were made with urea and sulphate. It was necessary to inject sodium sulphate in order to obtain a high enough plasma content for accurate analysis, and this substance was usually given in 6 p.c. gum arabic solution to retain the physiological colloid osmotic pressure. Urea, although normally present in the blood in sufficient quantity, was occasionally injected with the sulphate, for the purpose of raising its concentration well above any excretory threshold value which may exist.

Male rabbits anæsthetised with paraldehyde or urethane were used because they could be catheterised easily, and an abdominal incision was unnecessary. After a soft rubber catheter had been introduced, the bladder was emptied as completely as possible by pressure on the abdomen. Gum sulphate solution (10 p.c. anhydrous Na_2SO_4) was then injected into the jugular vein, and the diuresis which occurred during

this process served to wash out any residual urine entrapped between folds of vesical mucous membrane. When the injection was complete, one or two minutes were allowed to elapse, in order to ensure uniform distribution of the sulphate through the circulation. The bladder was once more completely evacuated, and then urine was collected for the minimum time which permitted the excretion of a convenient quantity for analysis.

The object of limiting the duration of the experiment was to avoid changes in concentration of plasma sulphate and urea during this period, which might result from dilution of the blood with tissue fluids, or from escape of sulphate into the tissues.

When enough urine had been obtained (usually five minutes), about three-fourths of the available blood was withdrawn from the carotid artery, and urea and SO_4 were estimated in the plasma and urine. It is the plasma content of any substance which represents the quantity available for excretion; and not, as Addis and his co-workers appear to have assumed in certain experiments(2), the amount present in the whole blood. It is not possible for the kidney to excrete the urea contained in the blood corpuscles or any other tissue, until this urea reaches it in solution in the plasma.

Since the relatively small amounts of SO_4 and urea excreted in the short time allowed could not materially alter the plasma content of these substances, the composition of the blood collected at the end of the experiment approaches very closely the average composition during the period of excretion. Rapid withdrawal of blood and rejection of the last portion prevents possible error from the dilution which accompanies hæmorrhage.

The plasma was separated by centrifugalisation. Urea was estimated by the urease method, the ammonia liberated being determined by vacuum distillation into standard ($N/60$) acid (Kruger—Reich—Schittenhelm), as this procedure was found to be more reliable than aeration. SO_4 was estimated in some cases gravimetrically, as BaSO_4 , and in others by the benzidine method. The possibility was recognised of loss of sodium sulphate by adsorption on coagulated protein, and, although it was considered that most of the sulphate could be recovered by repeated washing of the coagulum, complete destruction of the proteins by oxidation with potassium chlorate and hydrochloric acid, or by preliminary incineration of the plasma, was in most cases preferred.

The results obtained in this group of experiments are given in Table I.

TABLE I.

No.	Wt. of rabbit grms.	Injection c.c. $a = \text{Na}_2\text{SO}_4$ 10% $b = \text{Na}_2\text{SO}_4$ and urea	Vol. of urine c.c. per min.	% urea in plasma	% urea in urine	Urine urea/Plasma urea	% Na_2SO_4 in plasma	% Na_2SO_4 in urine	Urine Na_2SO_4 /Plasma Na_2SO_4	Concentration of SO_4 by kidney, Urea ≈ 1
1	2150	a 21.5	4.80	.0342	.0725	2.12	.2352	1.1738	4.99	2.35
2	2450	a 24.5	7.87	.0235	.0600	2.55	.2810	1.1512	4.10	1.61
3*	1750	a 17.5	0.80	.0780	.2143	2.75	.3096	2.3433	7.57	2.75
4*	1850	a 18.5	1.00	.0420	.1200	2.86	.4924	2.1178	4.30	1.50
5*	1430	a 7.2	0.50	.0712	.4200	5.90	.1238	1.6957	13.70	2.32
6*	2700	a 27.0	2.17	.0611	.1450	2.37	.3594	1.5399	4.28	1.80
7	2350	b† 23.5	2.30	.1920	.6750	3.51	.2206	1.5260	6.92	1.97
8	2050	b† 41.0	0.75	.1530	.9450	6.18	.2308	2.5704	11.14	1.80

* Paraldehyde.

† Na_2SO_4 10 %, urea 10 %.‡ Na_2SO_4 5 %, urea 5 %.

A glance at this table shows that in each case SO_4 has been concentrated by the kidney to a considerably greater extent than urea. In the examples given here, the increase in concentration of SO_4 during its passage from plasma to urine varies between 1.5 and 2.75 times that which urea undergoes in the same process. It is not possible to explain this difference by assuming that the only function of the tubules is the absorption of water or Locke's fluid from the glomerular filtrate; for in that case the plasma concentrations of the two substances multiplied by a common factor would give their concentrations in the urine. To retain the hypothesis of a purely absorptive mechanism one must, therefore, admit the absorption of urea by the tubule epithelium. Cushny (5) has already shown that urea may be taken up from the kidney, when the circulation is depressed and the secretion of urine has ceased, and Addis and Shevky (2) confirmed this by finding more urea in the renal vein than in the artery. This means that urea must be included in Cushny's group of "threshold" bodies (1), and suggests either that it is of some value to the organism, or that the normal kidney is not a perfect excretory apparatus. As an analogy, it should be remembered that CO_2 , although a waste product, is essential in preserving the physiological reaction of tissue fluids, and urea also may have some function as yet unrecognised; the suggestion may be hazarded that this function is of a physical nature and dependent on the ready diffusion of urea through the cells and fluids in the body.

On the theory of tubule secretion there is, of course, no reason why the epithelium should not secrete two substances at different degrees of concentration. If this view is held, however, it appears that the kidney has more difficulty in secreting urea than in secreting SO_4 . But urea

diffuses through living tissues more readily than SO_4 , and its secretion might, therefore, be expected to present less difficulty to the kidney. Diffusibility is a property which should aid secretion and absorption, however "vital" these processes may be; and it is fairly easy to understand why a heavy di-valent ion like SO_4 , which has reached the tubules in the glomerular filtrate, should offer more resistance to absorption by the epithelium than such a substance as urea; while it is not easy to see why a secreting cell should, apparently, have more difficulty in forcing out urea than SO_4 , unless its efficiency is increased by the toxicity of the substance with which it has to deal.

At any rate, whichever theory is held, there is little doubt that urea passes through the tubule cells in one or other direction. This statement is supported by the recent precipitation of a urea compound in small quantities in the protoplasm of these cells (3).

The relative concentration of urea in the blood and urine has been used in recent years as a test for the efficiency of the kidney (Amhard), and this is based on the view that urea is a "no-threshold" substance, that is, a substance all of which is excreted as rapidly as possible. But, since I have shown that the kidney tends to retain a certain proportion of the urea in the blood, this view must be revised.

Neglecting the possible absorption of SO_4 , the strengths of the solutions of urea absorbed can be calculated from the figures in the examples given above. They vary from .0117 p.c. to .0573 p.c. where no urea was injected, and give no support to the theory of absorption of a fluid of constant composition. The calculation is based on the assumption that the SO_4 excreted in a given time has passed through Bowman's capsule at its plasma concentration, and thus indicates the amount of glomerular filtrate. If the volume of the urine is subtracted from this, the remainder must represent the fluid absorbed. Urea, also, was filtered at its plasma concentration, and the quantity that reaches the urine is the difference between the theoretical output if urea had been concentrated to the same extent as SO_4 , and the amount absorbed by the tubule cells. The amount in the urine is known and the filtrate can be estimated; hence, the urea absorbed can be determined. This urea was dissolved in the calculated quantity of absorbed fluid. In this relation it may be added that these calculations reveal an extraordinary variation in the amount of glomerular filtrate per minute. I hope to return to this point in a later paper.

It will be observed that in some of the rabbits the plasma urea percentage was high (Nos. 3, 4, 5 and 6). In these cases paraldehyde

made to excrete against a pressure varying from 20 to 30 mm. of mercury, and the urine so obtained was compared with that excreted simultaneously by the other kidney, working under normal conditions. The urine from the pressure side was, of course, much smaller in amount than that from the normal side, and its solid constituents were present in greater concentration. As Cushny (1) has pointed out, this small increase in pressure could have no demonstrable effect on secretion pressure, for, if secretion takes place, the epithelium must be capable of overcoming the enormous osmotic resistance which crystalloids offer to their removal from the plasma. The reduction in the quantity of urine must result from neutralisation of part of the pressure (renal blood pressure) available for glomerular filtration; and if the tubule cells are able to absorb fluid, the efficiency of this process will be increased by prolongation of the period during which they can act.

Sulphate, urea, and phosphate were used for the experiments, and the method consisted in injecting a solution of two of these substances, and collecting the urine from each kidney separately, by means of capillary cannulæ introduced into the ureters. On one side the ureteral pressure was raised, either by connecting the cannula with a fine rubber tube provided with a clip and capillary manometer, or by using a long cannula, bent upwards near the point where it left the ureter, and having the vertical portion about 30-35 cm. in height. The volume of both these arrangements was quite small (about 0.3 c.c.), and by allowing six to eight drops of urine to escape, one could be certain that the fluid collected was really excreted against the indicated pressure. It was frequently found that before pressure was applied, the two kidneys were excreting urine at very different rates. This may be a normal condition in the rabbit, or may have been due to reflexes resulting from operative interference with the ureters. On examination, however, it was found that the urine from the two sides was almost identical in composition and concentration, at any rate as regards the substances injected, as the following example shows:

	Vol. per min.	% urea	% Na ₂ SO ₄	Urea/ Na ₂ SO ₄
Right ...	·357 c.c.	1·0700	2·1063	·5080
Left ...	·200 „	1·0900	2·1590	·5049

It is probable, therefore, that the difference in volume is to be explained by a difference in the rate of glomerular filtration; since if it were due to any alteration in secretion or absorption, the concentrations of the fluids excreted by the two kidneys could not be the same. It was necessary, also, to establish the fact that a temporary increase in

ureteral pressure does not injure the kidney; and in order to do this, some urine was obtained from a kidney which had, about a quarter of an hour previously, been subjected for 35 minutes to a pressure of 25-30 mm. of mercury. This was compared with the corresponding urine from the other side and analysis gave the following result:

	Vol. per min.	% urea	% Na_2SO_4	Urea/ Na_2SO_4	Urea per min.	Na_2SO_4 per min.
Right	·202 c.c.	·9700	2·4157	·4015	·00255	·00634
Left (after pressure)	·287 „	·8900	2·3120	·3849	·00250	·00665

The left kidney has not completely recovered in 15 minutes, but if this urine is compared with the fluid which it excreted during the time that the ureteral pressure was raised (Table IV, No. 2), it will be seen how rapidly the normal condition is being resumed. Hence the effect of a small increase in pressure is very transient, the derangement of renal function persisting for only a short time after the pressure is removed. There is probably slight congestion, with swelling of the tubule epithelium, and the kidney subsequently gets rid of the accumulated fluid by excreting an increased amount of rather dilute urine.

In most of the experiments the solution injected contained 5 p.c. of urea and 5 p.c. of sodium sulphate. It was usually necessary to give large quantities during the period of collection of urine, in order to maintain a good blood-pressure for a sufficient time to permit of 1-2 c.c. being excreted against pressure. In one experiment sulphate and phosphate were given, and in this case the solution was made more dilute (about 2 p.c. of each) owing to repeated failures to get enough urine from the pressure side with stronger solutions.

The results are given in Table IV.

TABLE IV.

Weight of rabbit grms.	Injection c.c. urea 5% Na_2SO_4 5%	Average pressure mm. Hg.	Vol. of urine c.c. per minute		Urea %		Na_2SO_4 %		Urea/ Na_2SO_4	
			Normal	Pressure	Normal	Pressure	Normal	Pressure	Normal	Pressure
2150	43	30	1·21	·16	·7250	·9750	1·4756	2·5069	·4913	·3889
2650	100	25	0·90	·11	·7700	·9024	1·8028	2·4038	·4271	·3754
2200	54	30	0·77	·07	·7150	·8300	1·7255	1·9516	·4144	·4253
2050	41	25	1·81	·72	·5000	·6425	1·1162	1·6838	·4479	·3816
2050	41	25	0·37	·20	·9450	·8600	2·5704	2·9070	·3676	·2958
					Na_2HPO_4 %				$\text{Na}_2\text{SO}_4/\text{Na}_2\text{HPO}_4$	
					Normal Pressure				Normal Pressure	
1400	Na_2SO_4 2% Na_2HPO_4 2%	22	0·52	·10	1·6864	2·2190	1·0421	1·3583	·6179	·6123

It will be seen that in addition to the decreased output of fluid caused by raising the ureteral pressure, the ratio of urea to sulphate has been

altered. The effect of resistance on the output of urea is greater than its effect on SO_4 . This is easy to explain on the absorption theory, if it is admitted that the tubule cells can take up urea. More time is available for absorption, and hence this process is carried further than on the normal side. From the point of view of secretion by the epithelium, it has been shown already that pressure can have no direct influence. One can assume, however, that it decreases the amount of fluid which is passing through the glomeruli. The tubule cells will, therefore, be compelled to secrete urea and SO_4 into a smaller bulk of fluid, and thus do their work against a higher osmotic pressure than would be the case if a more rapid stream of fluid were passing. This hindrance to secretion will, presumably, have most effect on the substance which is most difficult to secrete, namely, the urea. But it is evident from the crystalloid content (urea p.e. + Na_2SO_4 p.c.) of the urine from the two kidneys that the osmotic pressure in the obstructed tubules was never even doubled, and it is not likely that the resistance was ever high enough to interfere with the action of cells which are designed to effect great changes in concentration.

Reference to Experiment 3 in Table IV will show that here the increased pressure has had very little effect on the ratio of urea to sulphate. In this experiment a long time was allowed to elapse before the collection of urine was begun, and the vitality of the kidney must have been low. Hence, the "vital" part of the excretory process was probably inefficient, and the fact that the excretion of urea approaches more nearly to that of SO_4 suggests that this "vital" element must be one of absorption rather than of secretion.

The actual outputs of sulphate and urea can be calculated from the figures given in the table. They are, of course, much smaller on the pressure side, since less urine is excreted, but the difference between the outputs of the two kidneys is more marked as regards urea than sulphate, except in Experiment 3, where the normal/pressure output ratios are practically the same.

A comparison of the excretion of SO_4 and HPO_4 was made under similar conditions (Table IV, No. 6). It would be expected from the similarity of the renal concentration ratios (urine content/plasma content) for these substances that pressure would not alter very appreciably their relative concentrations in the urine. As will be seen by reference to the table, this was the result obtained, the difference being small enough to fall within the limits of experimental error.

SUMMARY AND CONCLUSIONS

No two substances were definitely proved to have exactly the same concentration ratio, but sulphate, phosphate, and creatinine differ only slightly in the degree to which their concentrations are raised, and a small relative alteration in their plasma concentrations during the experiment might account for this difference. These three substances are concentrated to a considerably greater extent than urea.

Raising the ureteral pressure increases the difference between the concentrations of urea and sulphate in the urine, by reducing the output of urea more than that of sulphate. The degree of difference between the concentration ratios of two substances seems to determine the extent to which pressure affects their relative concentrations in the urine.

The evidence obtained is considered, on the whole, more favourable to the theory of reabsorption in the tubules than of secretion, for the following reasons:

(a) The concentration ratios of certain very different substances are so nearly the same that it requires a stretch of imagination to suppose that they are secreted independently of each other. It is simpler to assume that they are concentrated by removal of water, and to account for possible slight differences in their concentration ratios by admitting that small quantities of "no-threshold" bodies may be absorbed.

(b) The greater the diffusibility of any of the substances examined the lower appears to be its concentration ratio. It is not easy to see why diffusibility should be a hindrance to secretion, but one can understand why it should aid absorption from the tubules.

(c) The results derived from experimental interference with excretion seem to support the absorption hypothesis.

Urea is absorbed in considerable amount, in the rabbit, even during the diuresis, about half of that which passes through Bowman's capsule is restored to the blood. It is probably a "threshold" substance, and may be of some value to the organism.

Possibly all substances are absorbed to a slight extent, but absorption in the case of "no threshold" bodies is negligible, and may be regarded merely as evidence of renal inefficiency.

The fluid absorbed cannot be constant in composition, for calculations based on the absorption theory show that the concentration at which urea is returned to the blood can vary within wide limits, although it never reaches the existing concentration of urea in the plasma.

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THE REGULATION OF THE GENERAL CIRCULATION RATE IN MAN. BY C. G. DOUGLAS AND J. S. HALDANE.

(From the Laboratory, Cherwell, Oxford.)

I. *Method of Investigation.*

IN conjunction with Dr Christiansen we described in 1914 a method of estimating the gas-contents of the mixed venous blood returning to the lungs, and at the same time measuring the rate of flow of the blood (1). We also gave data relating to the gas-contents and rate of flow during rest in several individuals. At the outbreak of war we were engaged in perfecting the new method and extending its application; but though one of us, who was not away on military service, was able, with kind help from Dr A. Mavrogordato, to continue part of the work and communicate a number of results to the Physiological Society in 1915, it was impossible, until lately, to complete what we had planned.

Our method consisted in using the whole of both lungs as an aerotonometer on Pflüger's principle, and thus ascertaining the gas-pressures of the mixed venous blood reaching the lungs. Knowing the gas-pressures of the venous blood, and knowing also the gas-pressures of the arterial blood from analyses of the normal alveolar air, we could calculate from the dissociation curves published in the previous part of our paper how much CO_2 a given volume of blood had taken up in passing round the circulation and how much oxygen it had lost. From a simultaneous determination of the respiratory exchange the total gain of CO_2 and loss of oxygen per minute could be ascertained. Hence the rate of blood-flow could be calculated in the same way as if the gas-contents of samples of blood from the right and left sides of the heart were determined directly, as in the method used by Zuntz and Hagemann for the horse, or the much simpler method recently used for the goat by Barcroft, Boycott, Dunn and Peters (2). The advantages, however, of experimenting on man, with all the physiological conditions under accurate control, and no anæsthetics or hampering legal restrictions, are evident.

In these experiments the subject expired deeply and then took a maximum breath of a suitable mixture (about 7.5 p.c.) of CO_2 and air.

After holding the breath four seconds, about half of it was expired through a tube arranged so that a sample of alveolar air was obtained by the Haldane-Priestley method. A second alveolar sample was then taken from the rest of the breath six seconds later. In a successful experiment the CO_2 -percentage in the two samples was practically the same, as the CO_2 -pressure in the alveolar air was in equilibrium with that in the oxygenated venous blood.

As we had shown, oxygenation of reduced or partially reduced defibrinated blood raises its CO_2 -pressure; and since the venous blood is oxygenated in the lungs when the method just described is used, the excess in CO_2 -pressure of the oxygenated venous blood over that of the arterial blood ought by calculation to be about 50 to 60 p.c. greater during rest than that of the unoxygenated venous blood. When, therefore, we found that the CO_2 -pressure of the oxygenated venous blood was 8 mm. higher than that of the arterial blood, the calculated CO_2 -pressure of the venous blood before oxygenation was only about 5 mm. higher, as can easily be seen from Fig. 3 of the paper. To measure directly the true CO_2 -pressure of the venous blood before oxygenation it was evidently necessary to inhale a gas-mixture such that both the oxygen-pressure and CO_2 -pressure of the venous blood were in equilibrium with those of the gas-mixture in the alveoli. The circulation rate, as calculated from the oxygen-pressure, could then be used to check the result calculated from the CO_2 -pressure. We mentioned that the circulation rate (an unexpectedly rapid one) calculated from the CO_2 -pressure was confirmed by calculation from the observed oxygen-pressure.

The oxygen-pressure experiments revealed a source of fallacy which became specially evident when we attempted to determine the venous oxygen-pressure just after forced breathing. As was pointed out by one of us in 1915 (3), the "alveolar air" as obtained by the Haldane-Priestley method represents the contents of the alveoli of the "air-sac" system described by Miller (4). Besides the air-sac alveoli which make up by far the greater part of the lung alveoli there are the alveoli of the respiratory bronchioles, alveolar ducts, and atria. These act as air-passages to the air-sac alveoli, and expand with the air-sac alveoli during inspiration. When a deep breath is taken of a mixture differing considerably in composition from the air-sac air, this mixture fills the air-passage alveoli and mixes with the air in the air-sac alveoli. But the mixture left in the air-sac or deep alveoli must differ in composition from that in the air-passage alveoli. Hence, apart from the effects of gaseous interchange between blood and air, the first portion of deep alveolar

air expired is seriously diluted with the air in the air-passage alveoli, and is thus nearer in composition to the mixture inhaled than the second portion.

At the end of a period of forced breathing the alveolar air contains nearly 20 p.c. of oxygen and only about 2 p.c. of CO_2 . When, in the endeavour to ascertain the gas-pressures of the venous blood after forced breathing, a single deep inhalation was made of a mixture containing about 4 p.c. of oxygen and 6 p.c. of CO_2 , it was found that the oxygen-pressure in the second alveolar sample usually rose higher than in the first, or failed to fall, even though the venous oxygen-pressure thus apparently indicated was incredibly high. Thus in one experiment the first alveolar sample gave 6.91 p.c. of oxygen, and the second 8.01 p.c.; and in another experiment the first sample gave 10.32, and the second 10.33 p.c. of oxygen. A similar incredible result was obtained when we endeavoured to determine the venous oxygen-pressure after quietly breathing for two minutes a mixture containing 26 p.c. of oxygen. The first alveolar sample contained 8.53, and the second 8.80 p.c. of oxygen. In a further experiment five deep and rapid breaths of air were taken, so as to raise the alveolar oxygen to nearly 20 p.c., and lower the CO_2 to about 2 p.c. A single deep breath of the mixture (about 4 p.c. of oxygen and 6.3 p.c. of CO_2) was then taken, with the result that the first alveolar sample contained 5.92 p.c. of CO_2 and 9.57 p.c. of oxygen, while the second contained 4.20 p.c. of CO_2 and 12.48 p.c. of oxygen. Thus the venous CO_2 -pressure was apparently less than 4.2 p.c. of CO_2 and more than 12.5 p.c. of oxygen; whereas, as numerous later experiments showed, the real venous CO_2 -pressure was about 6.3 p.c., and the oxygen-pressure about the same. On immediately repeating the last experiment, but with two deep breaths of the mixture, so as to wash out the air-sac alveoli more thoroughly, the first alveolar sample gave 5.87 p.c. of CO_2 and 7.35 p.c. of oxygen, while the second gave 6.11 p.c. of CO_2 and 6.65 p.c. of oxygen. This experiment indicated correctly that the venous CO_2 -pressure was over 6.11 p.c., and the oxygen-pressure under 6.65 p.c.

It was quite evident from these experiments that by merely taking a single deep breath of a gas-mixture it is impossible to produce an even mixture in the lung alveoli. The same fact has been pointed out very clearly by Sonne (3). When, however, there is no great relative difference between the percentage of a gas in the mixture inspired and the percentage already present in the alveolar air, this source of fallacy is of minor importance. If, for instance, the mixture contains 6.6 p.c. of

CO₂, the pre-existing alveolar air 5.6 p.c., and the (true) venous CO₂-pressure corresponds to 6.3 p.c. of CO₂, no serious error in measuring the venous CO₂-pressure can arise from uneven mixture in the lungs. In measuring the venous oxygen-pressure, however, or the CO₂-pressure when it is considerably above the existing alveolar CO₂-pressure, there is necessarily a large difference between the percentage in the mixture inspired and that in the normal alveolar air, so that it is essential to ensure proper mixture by taking more than one deep breath of the mixture. We found that with a large difference in the percentages, as in measurements of venous oxygen-pressure, at least three maximal breaths are required.

In the previous paper we gave data, calculated from the observed CO₂-pressure of the oxygenated venous blood in the lungs, showing that for three male subjects the CO₂-pressure of the venous blood before oxygenation, after ten minutes' rest on a chair, was about 5 mm. above the arterial CO₂-pressure. Calculating from the dissociation-curve given in Fig. 3 of the paper, the extra charge of CO₂ per 100 c.c. of venous blood was only 3.15 to 3.3 c.c., or 21 to 22 p.c. of the extra charge which would be present if all the oxygen of the arterial blood had been utilised for CO₂-production. This gave a calculated circulation rate of about 7.7 litres per minute, which was much higher than the rate given during rest by the nitrous oxide method.

In order to check our figures we proceeded, in 1914, to determine directly both the true CO₂-pressure and the true oxygen-pressure of the venous blood. Many of the earlier experiments were, however, unsatisfactory, as only one preliminary breath was employed, and it was not until we employed three breaths that consistent results were obtained. Various preliminary experiments were also required in order to discover the proper proportions of both oxygen and CO₂ in the gas-mixture to be employed. It is evident that unless both of these proportions are about right it is impossible to measure directly and accurately either the true oxygen-pressure or the true CO₂-pressure of the venous blood.

It might seem that on account of the slowness with which the gas-pressures in the alveolar air and venous blood equalise themselves it would be better to hold the breath for a longer period than five or six seconds between the two alveolar samples. We tried longer periods by taking a third sample a few seconds after the second, but the results seemed to indicate that the delay gave time for some of the imperfectly oxygenated blood leaving the lungs to pass round the circulation and return. In several experiments there was an abrupt drop in the oxygen-

percentage, but no corresponding rise in the CO_2 percentage, of the third sample. We concluded from this observation that even during rest the whole experiment ought to be terminated within about 15 or 16 seconds from taking the first breath of the gas mixture. Our results indicated that a volume of blood equal to that in the whole body may pass through the lungs in about 35 seconds during rest. It is therefore only to be expected that some of the blood will complete the round of the circulation during considerably less than this period. As hardly any dissociable oxygen is stored in the tissues the effects on the venous oxygen pressure of blood which has had time to come round will show themselves at once, whereas the venous CO_2 pressure will be affected more slowly, since the tissues have a far greater storage capacity for dissociable CO_2 .

Accurate determinations of the CO_2 pressure in oxygenated venous blood is easier than the simultaneous determination of pressure of oxygen and CO_2 in unaltered venous blood, but a small error in determining the arterial CO_2 pressure tells heavily in calculations of the circulation rate from arterial and venous CO_2 -pressures, since the difference is so small, particularly during rest. An error in determining arterial CO_2 pressure is, however, considerably less serious when the calculation is based on the CO_2 pressure of oxygenated venous blood. Simultaneous determinations of the oxygen and CO_2 pressures of venous blood are nevertheless needed for the purpose of checking the accuracy of the method.

In calculating from the venous oxygen pressure how much of its charge of oxygen the mixed venous blood returning to the lungs has lost, we must know the dissociation curve of the oxy-haemoglobin in the blood. This curve, however, as Barcroft has shown (6), varies considerably in different individuals. Our experiments on venous oxygen pressure have been made on ourselves, and our own dissociation curves have been carefully observed and do not differ sensibly. They are given for venous blood in Fig. 4 of the previous paper. For other persons it would be necessary to determine the dissociation curves if accurate calculations were to be made from the venous oxygen pressure, of the oxygen lost by a given volume of blood in passing round the circulation. The dissociation curves of blood for CO_2 vary also to some extent in different normal individuals and for the same individual at different phases of digestion, but so far as our own experience goes, and that of others who have repeated by reliable methods our experiments on the dissociation curves of human blood for CO_2 , the different curves run almost parallel to one another in normal persons, so that within the limits of difference in CO_2 pressure of arterial and venous blood it is easy to calculate suffi-

ciently closely the extra volume of CO_2 absorbed for a given rise of CO_2 -pressure.

The method which we finally adopted is first to obtain from a pilot experiment the approximate composition of the gas-mixture required to give equilibrium with the venous gas-pressures. A mixture of this composition is then prepared in a Douglas bag with the help of two or more gas meters, and two or three experiments made with it, varying somewhat the depths of the preliminary inhalations, so that the gas-percentages in the alveolar air come slightly higher or lower in the different experiments, and are thus slightly above or below the venous gas-pressures. The actual venous gas-pressures are thus "straddled," as in sighting shots with a gun, and can then be calculated from the rises or falls of the gas-pressures in the second alveolar samples as compared with the first. As an alternative and more satisfactory method, particularly when only the CO_2 -pressure of the oxygenated venous blood is being determined, three bags of mixture are prepared, with differences of about 0.5 p.c. of CO_2 between them, and an experiment is made with each bag.

In making an experiment a deep expiration is first made, followed instantly by a deep inspiration from the bag, another deep expiration to air and inspiration from the bag, and a final deep expiration to air and inspiration from the bag. The last inspiration is held for two seconds, and a sample of alveolar air taken at the end of a sharp expiration of about 1600 c.c. We regulated the depth of this expiration by attaching to the end of the expiratory tube the adjustable "concertina" of the apparatus figured by Haldane, Meakins and Priestley, vol. 52, p. 438 of this Journal. The concertina was detached from the rest of the apparatus, inverted, and set so as to hold just 1600 c.c. As soon as the first sample was taken the concertina was released, and after five seconds a second alveolar sample was taken with a maximum expiration, so that about 3500 c.c. in all had been expired into the concertina.

Just after or before the experiment, and with the subject remaining under exactly the same conditions, the metabolism is determined with all the usual precautions by the Douglas bag method. Samples of alveolar air for the determination of arterial CO_2 -pressure are taken as nearly as possible at the same time and under the same conditions as the samples for venous gas-pressures. We usually took one pair just before, and one pair just after, the samples for the venous gas-pressures. To avoid the oscillations in alveolar, and therefore in arterial, CO_2 -pressure which, as Dodds⁽¹⁾ has recently shown, are caused by the digestive secretions

following a meal, the experiments were made some hours after a meal had been taken.

We adhered to the Haldane-Priestley method of determining directly the CO_2 -pressure of the alveolar air, and inferring from this the CO_2 -pressure of the arterial blood. The method of determining by an indirect calculation the CO_2 -pressure of alveolar air has, as is well known, been adopted and vigorously defended by Krogh and Lindhard (8). They contend that the effective dead space in breathing corresponds simply to the capacity of the respiratory passages down to where alveoli begin, and is constant or varies but little, whether the breathing is deep or shallow, so that when once this space has been determined it is possible to infer the mean percentage of CO_2 in the alveolar air from the mean percentage of CO_2 in the expired air and the mean depth of breathing. Thus if the mean percentage of CO_2 in the expired air were 3.8, the mean depth of breathing 450 c.c., and the dead-space, including that of the mouth-piece, 150 c.c., the mean percentage of CO_2 in the alveolar air would be $\frac{3.8 \times 450}{450 - 150} = 5.7$. They therefore infer that the mean arterial CO_2 -pressure corresponds to this calculated percentage.

It is quite probable that the capacity of the respiratory passages down to the terminal bronchioles varies but little in any individual during health; and in other respects the reasoning of Krogh and Lindhard is quite logical. We must point out, however, that if the reasoning were in fact correct it would upset completely the theory of the regulation of breathing in accordance with the CO_2 -pressure or pH of the arterial blood. Haldane and Priestley showed experimentally that the breathing is regulated from minute to minute in accordance with the alveolar CO_2 -pressure as measured *directly* by the now well-known method which they introduced. They also showed that this was so whether the breathing was deep or shallow, and still more striking evidence of this was given by Haldane in the above-mentioned paper. Now with deep breathing, as during muscular work, or when the frequency of breathing is voluntarily diminished, the alveolar CO_2 -pressure is far lower when calculated on Krogh and Lindhard's assumptions than when determined directly. Hence if both Krogh and Lindhard's assumptions, and the theory that the breathing is regulated by the CO_2 -pressure of the alveolar air and arterial blood, were correct, the subject would become apnoeic. As he does not become apnoeic, either during muscular exertion or during voluntary slow breathing, the choice lies between abandoning either Krogh and Lindhard's method of calculating the arterial CO_2 -pressure, or the theory of regulation of

breathing by the pH of the blood. We had no hesitation in adhering to the direct method of Haldane and Priestley. For a fuller discussion of this subject, and of the misconceptions which, we think, are involved in Krogh and Lindhard's reasoning, we may refer to Chapter II of the book on Respiration (now passing through the Press) by J. S. Haldane.

II. Test experiments.

A series of experiments were made expressly to test the assumptions on which the method is based, and more particularly to ascertain whether the CO_2 -combining capacity of the blood in the living body is affected in the same way as fresh defibrinated blood outside the body when the degree of saturation of the hæmoglobin with oxygen is varied. Parsons⁽⁹⁾, Yandell Henderson and Haggard⁽¹⁰⁾, Peters and Van Slyke⁽¹¹⁾, and others have verified with defibrinated blood the results in this respect which were published in the paper by Christiansen, Douglas and Haldane; but Henderson and Haggard found that oxygenation had no influence on the CO_2 -combining capacity of oxalated blood. Peters and Van Slyke found, on the other hand, that in oxalated blood oxygenation had the same influence as on defibrinated blood. Perhaps the apparent contradiction depends on the amount of oxalate added. In view of the doubt as to whether coagulation does not alter the effects of oxygenation on the CO_2 -combining capacity of blood, it seemed essential to test the matter *in vivo*.

These experiments are given below fully in Table I, as they illustrate well the details of our method. The preliminary pilot experiment is also included. In order to obtain sufficiently accurate results it was necessary to have a fair difference between arterial and venous CO_2 -pressures; and as the difference increases to a very marked extent with the general metabolism, we increased the metabolism to a moderate extent by making the subject perform a fixed amount (103 kgm. per minute) of very gentle work on a Martin's ergometer during the experiment and (as in all other work experiments) for at least ten minutes before making any observations. The arterial CO_2 -pressure and metabolism were determined in the usual way.

It will be seen how close the agreement is in individual determinations of the excess of venous over arterial CO_2 -pressure or of venous oxygen-pressure, even on different days. In all our experiments there was the same consistency in the results for the same individual under the same physiological conditions, though different individuals might give very distinctly different results.

TABLE I. DOUGLAS. Work of 163 kg.m. per minute.

Date	Barometer mm. Hg.	Mixture in bag		First alveolar sample		Second alveolar sample		Approximate venous gas pressure		Alveolar CO ₂ % during experiment	Excess of venous over arterial CO ₂ pressure	Respiratory exchange per minute in c.c. at s.r.p.		Respira- tory quotient	Pulse- rate per minute
		CO ₂ %	O ₂ %	CO ₂ %	O ₂ %	CO ₂ %	O ₂ %	CO ₂	O ₂			O ₂	CO ₂		
14/4/21		5.95	5.23	6.63	6.49	6.68	6.12	—	—	5.53	—	—	—	—	84
(Pilot experiment)		"	"	6.51	6.61	6.72	6.00	—	—	—	—	—	—	—	—
15/4/21	749	7.11	3.96	6.91	4.76	6.91	4.88	0.91 { 0.98	5.00 { 4.79	5.62 (39.4 mm.)	1.36 (9.6 mm.)	640	572	0.894	75
		"	"	6.90	4.84	6.97	4.71	7.04 { 49.0 mm.)	4.58 { (33.6 mm.)	—	—	—	—	—	—
		7.00	ca. 19.5	6.99	—	7.25	—	7.51 { 7.53	—	5.69 (39.0 mm.)	1.89	623	502	0.805	81
		7.87	"	7.46	—	7.52	—	7.58 { (53.2 mm.)	—	—	—	—	—	—	—
		8.35	"	7.80	—	7.72	—	7.64 {	—	—	—	—	—	—	—
16/4/21	750	7.32	3.95	7.04	4.70	6.95	4.78	6.80 { 6.83	4.86 { 4.90	5.33 (37.5 mm.)	1.50 (10.5 mm.)	608	530	0.871	76
		"	"	6.89	5.16	6.82	5.05	6.75 { (48.0 mm.)	4.94 { (34.5 mm.)	—	—	—	—	—	—
		7.42	ca. 19.4	6.74	5.92	6.81	5.70	0.83	—	—	—	—	—	—	—
		7.72	"	7.30	—	7.47	—	7.64 { 7.68	—	5.69 (40.0 mm.)	1.99 (14.0 mm.)	655	553	0.845	77
		8.41	"	7.41	—	7.51	—	7.61 { (54.0 mm.)	—	—	—	—	—	—	—
		"	"	7.92	—	7.86	—	7.80 {	—	—	—	—	—	—	—
18/4/21	754	7.55	"	7.31	—	7.48	—	7.05 { 7.75	—	5.37 (38.0 mm.)	2.38 (16.8 mm.)	637	586	0.921	81
		7.89	"	7.35	—	7.56	—	7.77 { (54.8 mm.)	—	—	—	—	—	—	—
		8.36	"	7.78	—	7.81	—	7.84 {	—	—	—	—	—	—	—
		7.13	3.79	6.87	4.72	6.75	4.69	0.03	4.60 { 4.93	5.29 (37.4 mm.)	1.36 (9.6 mm.)	615	536	0.872	80
		"	"	6.92	4.69	6.77	4.82	0.65	4.95 { 4.91	—	—	—	—	—	—
		"	"	6.85	4.93	6.75	4.92	6.05 { (47.0 mm.)	5.20 { (34.8 mm.)	—	—	—	—	—	—
		"	"	6.80	5.22	6.75	5.21	6.70 {	—	—	—	—	—	—	—
		Mean values for oxygenated venous blood													
						7.67 (54.0 mm.)		6.82 (48.0 mm.)	4.87 (34.8 mm.)	5.58 (39.3 mm.)	2.09 (14.7 mm.)	638	547	0.857	80
		Mean values for unoxygenated venous blood													
						6.82 (48.0 mm.)		4.87 (34.8 mm.)	5.41 (38.1 mm.)	—	1.41 (9.9 mm.)	621	546	0.879	77

* As the high percentage of oxygen in the first alveolar sample indicated very imperfect washing out of the deep alveoli, the venous oxygen pressure could not be calculated.

It will also be seen from Table I that when the venous blood was oxygenated the mean difference in CO_2 -pressure between alveolar air or arterial blood and venous blood was 14.7 mm., as against only 9.9 mm. when the venous blood was in its natural state before oxygenation. The excess is 48.5 p.c. There can thus be no shadow of doubt that in the living body oxygenation raises the venous CO_2 -pressure, and promotes

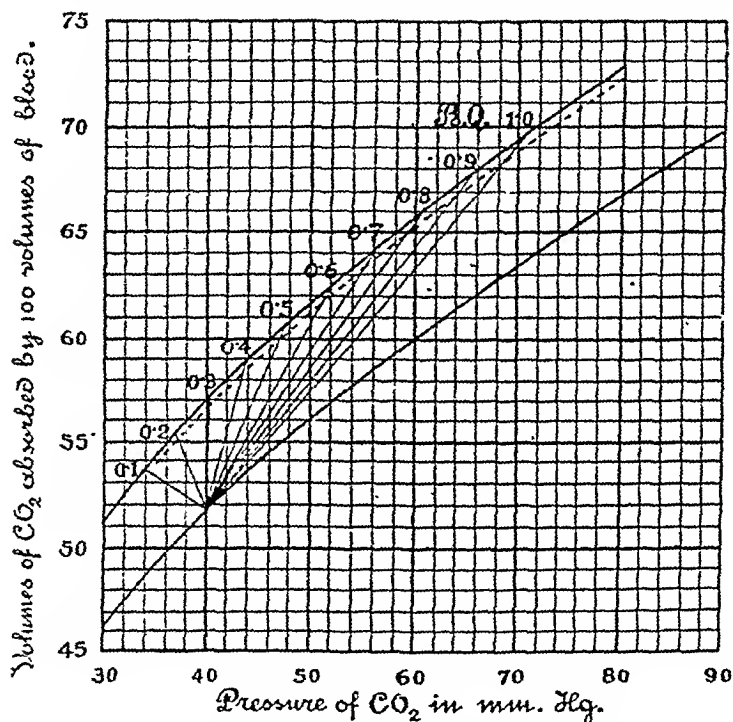


Fig. 1. Graphic representation of relation between CO_2 -pressure and CO_2 -content of blood in the living body with varying respiratory quotients.

The upper curve represents the relation when the blood is completely deprived of oxygen; and the lower curve when the hæmoglobin is 95 p.c. saturated with oxygen, as in average normal arterial blood. The thin lines joining the two curves represent the relations in the living body with varying respiratory quotients. It is assumed that the blood has the normal hæmoglobin content, equivalent to 18.5 p.c. oxygen capacity. before mentioned dotted line shows how the upper curve is altered when, as in the experiments on lism were, the blood contains only 92 p.c. of the normal concentration of hæmoglobin.

will be seen of CO_2 into the alveolar air, in the manner which we in-
 cess of previous paper from experiments with defibrinated blood
 on a

in the closely the results agree with what can be calculated
 ns defibrinated blood it is necessary to take into con-
 tory quotient; during the experiments. Fig. 1,

constructed from the same data as Fig. 3 of our former paper, but allowing for the fact that the arterial blood is only about 95 p.c. saturated with oxygen¹, shows graphically the relations between CO_2 -pressures in oxygenated and unoxygenated venous blood with varying respiratory quotients. It will be seen at once how much the relations vary with the quotients. Thus with a quotient of 0.7 and an excess charge in the venous blood of 5 volumes of CO_2 per 100 volumes of blood, the calculated excess of CO_2 -pressure in the venous blood is 80 p.c. greater on oxygenation than before oxygenation; whereas with a quotient of 1.0 the corresponding excess is only 37 p.c. As the respiratory quotient falls the relative difference increases, until with a quotient of about 0.3 it becomes infinity, since, as shown in Fig. 1, there is no excess in CO_2 -pressure until the venous blood is oxygenated. Of course, however, a quotient as low as 0.3 is only possible temporarily under highly artificial conditions, as after forced breathing, or when air containing an excess of CO_2 is breathed. With a quotient of less than 0.3, but still positive, CO_2 passes out by diffusion into the alveoli although the CO_2 -pressure of the venous blood before oxygenation is less than that of the alveolar air.

It will also be seen from the figure that the relative difference increases with increase of the excess charge of CO_2 in the venous blood. Thus with an excess charge of five volumes of CO_2 and a quotient of 0.9 the calculated excess of CO_2 -pressure is about 40 p.c. greater in oxygenated venous blood than before oxygenation: whereas with the venous blood completely deprived of oxygen and with the corresponding charge of 16 volumes of CO_2 , the calculated excess of CO_2 -pressure is about 71 p.c. greater in oxygenated venous blood than before oxygenation.

The thin lines in Fig. 1 are not perfectly, though very nearly, straight. The deviation from straightness is due to the fact that the dissociation curve for oxygenated blood is not quite parallel to that for de-oxygenated blood.

During the experiments shown in Table I the respiratory quotient varied from 0.80 to 0.92. Hence the different pairs of experiments are not strictly comparable with one another as regards the differences of CO_2 -pressure caused by oxygenation. The mean respiratory quotient was .022 lower for the experiments with oxygenated blood. This makes the mean difference in calculated rise about 3 p.c. greater than if the quotients had been equal. After making the corresponding correction the excess (calculated from Fig. 1) of venous above arterial CO_2 -pressure

¹ This allowance raises slightly the level of the curve for oxygenated venous blood.

III. *Venous gas-pressure and circulation rate during rest and muscular work.*

We approached the investigation under the impression, derived from the results of Zuntz and Hagemann on the horse, and Krogh and Lindhard on man, that the gas-contents of mixed venous blood do not on the whole differ very greatly during rest and activity. Even in our earlier experiments it became evident, however, that the CO_2 -content is much higher and the oxygen-content much lower, during muscular activity. Nevertheless we failed for long to realise that a quite small increase in the metabolism of complete rest has a distinct influence on the venous gas-contents. The most complete and careful series of experiments is one made since the war with C. G. D. as subject. The rest experiments were made in either the sitting or recumbent position, after resting for about half an hour. The work experiments were made during measured work on a Martin ergometer. The results are summarised in Tables II A and II B. Owing to the cost of printing, only average results are given. The figures in brackets in Table II A represent gas-pressures calculated (taking into account the respiratory quotients) as distinguished from gas-pressures directly determined.

It will be seen at once that as the metabolism increased the difference between arterial and venous CO_2 -pressure or oxygen-pressure increased also. The change was, however, much more marked at low than at high metabolisms. Thus with an increase in metabolism of about 400 c.c. per minute from the basal rate of 233 c.c. per min. there was an increase in the difference between arterial and oxygenated venous CO_2 -pressure of 9.3 mm.: whereas there was only an increase of 2.2 mm. in the difference when the metabolism increased by nearly 600 c.c. per min. from 1390 to 1974 c.c.

Fig. 2 represents graphically some of the chief results. It will be seen from this figure and Tables II A and II B that the blood-flow was about seven to eight litres per minute during rest, and increased very little when the metabolism was increased three times. With further increase in the metabolism the circulation rate increased more and more, however, until finally it was increasing in almost direct proportion to the metabolism. Very roughly speaking, the circulation rate increased *pari passu* with the pulse rate, and the output of blood per heart-beat remained nearly constant at about 125 c.c. A maximum output per beat was, however, obtained during rest, with the metabolism at its lowest. The percentage utilisation of the arterial oxygen rose from about 19

with the lowest metabolism to 64 with a metabolism 8.5 times as great. There was thus an increase of 3.3 times in the percentage utilisation,

TABLE IIA. DOVOLAS. Weight 66 kils.; haemoglobin percentage about 02 (Haldane scale).

	Number of determina- tions of venous gas pressure	CO ₂ -pressure of mixed venous blood in mm. Hg		Oxygen pressure of mixed venous blood in mm. Hg		Alveolar CO ₂ -pressure in mm. Hg	Excess of venous over arterial CO ₂ -pressure in mm. Hg	
		Oxygenated	Unoxy- genated	Observed	Calculated		Oxygenated	Unoxy- genated
Lying down after fasting for 15 hours	8	(45.8)	44.0	40.1	47.1	40.0	(5.2)	3.4
Sitting upright on chair ..	5	(46.9)	44.8	46.0	44.8	40.1	(0.8)	4.7
Sitting upright on chair after fasting for 8 hours	14	45.8	(43.7)	—	47.5	40.2	5.6	(3.5)
Ergometer, 103 kg m. per min.	10	(52.5)	48.0	34.8	35.7	38.1	(14.4)	9.0
Ergometer, 264 kg m. per min.	9	54.0	(49.0)	—	35.3	39.3	14.7	(9.7)
Ergometer, 512 kg m. per min.	24	57.2	(51.5)	—	32.7	41.1	16.1	(10.4)
Ergometer, 752 kg m. per min.	11	62.0	(54.8)	—	28.1	41.0	21.0	(13.8)
Ergometer, 870 kg m. per min.	12	65.3	(50.8)	—	20.2	41.5	23.8	(15.3)

TABLE IIB. DOVOLAS.

	Respiratory exchange in c.c. per minute at s.r.p.		Respiratory quotient	Pulse rate per minute	Blood flow per minute in litres		Output of heart per beat in c.c.	Percentage utilisation of arterial oxygen	
	O ₂	CO ₂			From CO ₂	From O ₂		From CO ₂	From O ₂
Lying down after fasting for 15 hours	233	180	0.77	53	7.5	6.8	135	19.0	20.0
Sitting upright on chair ..	205	248	0.84	66	8.0	8.4	124	22.5	21.4
Sitting upright on chair after fasting for 8 hours	256	200	0.78	50	8.0	—	135	19.5	—
Ergometer, 103 kg m. per min	621	540	0.88	77	8.8	8.5	112	43.1	44.5
Ergometer, 264 kg m. per min.	638	547	0.86	80	8.8	—	110	44.0	—
Ergometer, 512 kg m. per min.	801	744	0.835	88	10.0	—	124	49.6	—
Ergometer, 752 kg m. per min.	1390	1215	0.875	114	13.8	—	121	61.3	—
Ergometer, 870 kg m. per min.	1074	1775	0.90	137	18.7	—	136	64.2	—
Ergometer, 870 kg m. per min.	2415	2370	0.98	170	—	—	—	—	—

and 2.6 times in the pulse-rate. So large an increase in percentage utilisation has not been observed in experiments on the blood-gases of animals,

probably because of the difficulties in securing normal resting and working conditions while the samples from the right ventricle were being taken.

Fig. 3 shows graphically the relation between metabolism and oxygen-intake per heart-beat. It will be seen that the curve for C. G. D. rises steeply at first with increasing muscular exertion, but finally reaches a horizontal asymptote with increasing heavy exertion. This shape of curve corresponds to the similar curves published by Yandell Henderson and Prince (13). The exact significance of the curve in the case

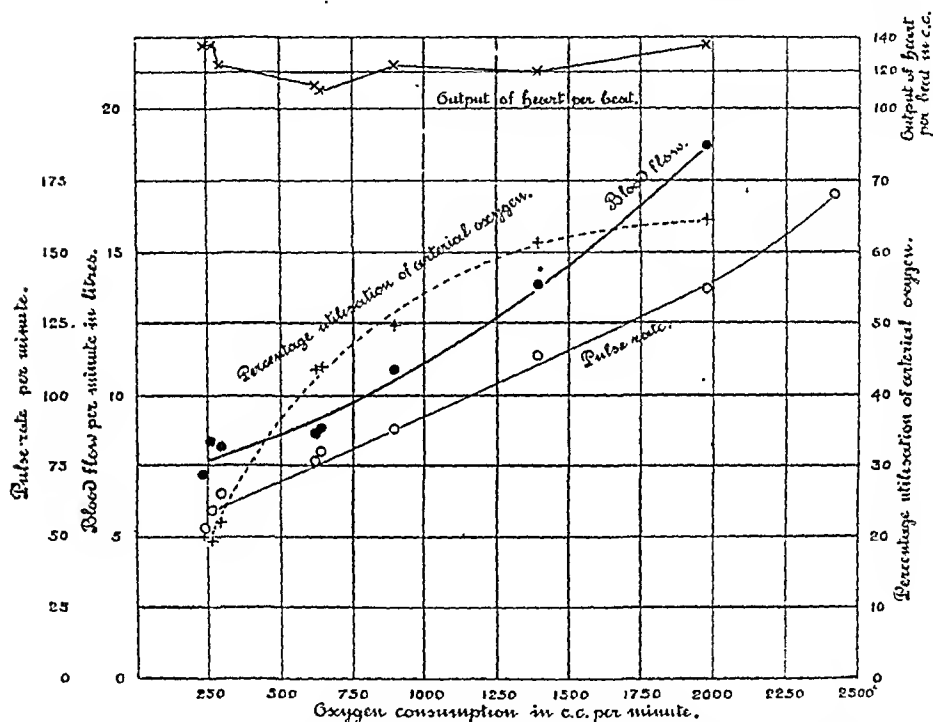


Fig. 2. Graphic representation of relations between blood-flow, percentage-utilisation of arterial oxygen, pulse rate, and output of the heart per beat with varying general metabolism in the experiments on Douglas.

of Douglas can now be seen. The rise, gradually tailing off, corresponds to the rise in percentage utilisation. For the last point plotted for C. G. D. we have no data for percentage utilisation; but to judge from the second last point (corresponding to the last point in Fig. 2) there would be little or no increase in percentage utilisation or in output of blood per heart-beat with the hardest work capable of being continued, and the further increase in pulse rate would be proportional to the increased metabolism. The circulation rate would thus be about 24 litres

per minute as compared with eight during rest. The pulse rate, as shown in Table II B, would be increased 3.2 times, and the oxygen-consumption 10.4 times.

Various other subjects besides C. G. D. were partially investigated. In the previous paper it was shown that in J. S. H. and J. G. P. there was during rest an excess of venous above arterial CO_2 -pressure similar to that in C. G. D. For J. S. H. we have numerous further observations showing that during rest sitting, not only the rise in venous above arterial CO_2 -pressure, but also the fall in venous oxygen-pressure, were about

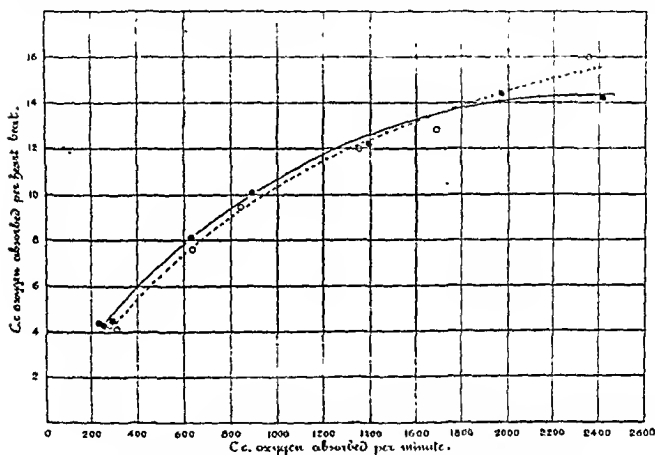


Fig. 3. Oxygen consumption per heart-beat with varying metabolism.
Continuous line, Douglas. Dotted line, Adolph.

the same as in C. G. D. Other determinations show also a similar behaviour of the circulation during work to that in C. G. D. These observations were, however, for the most part incomplete in one way or another and need not be quoted in detail. In another subject, J. B. S. H., the resting excess of CO_2 -pressure in the oxygenated venous blood was found to be practically the same as in the previous three subjects. We found, however, two subjects, Messrs Adolph and Peskett, in whom there was a different resting value, and we are indebted to them for acting as subjects for further experiments. Table III summarises the results obtained.

TABLE III.

Number of determinations of venous CO ₂ pressure	CO ₂ pressure of mixed venous blood in mm. Hg.			Excess of venous over arterial CO ₂ pressure in mm. Hg.		Respiratory exchange in c.c. per minute at s.r.p.		Pulse rate per minute	Blood flow per minute in litres	Output of heart per beat in c.c.	Percentage utilisation of arterial oxygen	
	Oxygen-ated, observed	Oxygen-ated, calculated	Alveolar CO ₂ pressure in mm. Hg.	Oxygen-ated, observed	Un-oxygen-ated, calculated	O ₂	CO ₂					
ANOLPH. Weight, 70 kilos.; hæmoglobin percentage about 100 (Haldane scale)												
sitting upright on chair* ...	15	51.4	48.0	40.0	10.8	7.4	309	268	0.87	76	5.8	30.0
Ergometer												
102 kg.m. per min.	—	—	—	—	—	—	634	727	1.15	84	—	—
264 kg.m. per min.	12	62.7	58.1	44.8	17.9	13.3	839	872	1.04	88	11.7	40.5
537 kg.m. per min.	8	70.6	63.6	47.2	23.4	16.4	1348	1365	1.01	112	14.4	52.5
774 kg.m. per min.	—	—	—	—	—	—	1688	1729	1.02	132	—	—
939 kg.m. per min.	—	—	—	—	—	—	2350	2370	1.01	147	—	—
PESKETT. Weight, 58 kilos.; hæmoglobin percentage about 100 (Haldane scale)												
sitting upright on chair* ...	5	52.1	48.2	40.0	12.1	8.2	279	241	0.865	70	4.7	33.4
Ergometer												
705 kg.m. per min.	5	69.8	61.2	45.0	24.8	16.2	1653	1535	0.93	102	15.5	59.6

* Not fasting.

* Not fasting.

It will be seen that in both subjects the excess of venous above arterial CO_2 -pressure during rest was considerably greater than in the subjects already referred to. The percentage utilisation of oxygen was therefore correspondingly greater, and in Peskett was as high as 33. In the case of Miss Christiansen, for whose venous CO_2 -pressure data were given in the previous paper, there was a similar high percentage utilisation. Corresponding with the high percentage utilisation there was, allowing for differences in the weights of the different subjects, not only a low circulation rate during rest, but also a low delivery of blood per heart-beat. During work, however, the delivery of blood per heart-beat increased considerably along with the increase in pulse rate, so that the circulation rate during considerable exertion became about as great as in the other subjects. In Peskett the output per heart-beat was apparently more than doubled during hard work, as compared with rest sitting, while in Adolph there was also a considerable increase. We must point out, however, that the figures for Adolph and Peskett are not so reliable as those for Douglas. The number of observations was sometimes insufficient to give more than approximate figures. It will also be noticed that in the case of Adolph the respiratory quotient during work was very suspiciously high; and if, as seems probable, this was due to some abnormal increase of breathing while the metabolism was being determined, there will be a corresponding error in the calculations.

In Douglas the circulation rate was nearly in proportion to the pulse rate. This corresponds to the conclusion reached in 1906 by Yandell Henderson from plethysmograph records of the heart-beats in dogs (14). It has been inferred that Henderson's conclusions must have been mistaken, since, assuming as has hitherto been done that the mixed venous blood loses at least a third of its oxygen during rest, an increase to ten times or more in the metabolism during work could not possibly be brought about without a very large increase in the output per beat. The figures for Douglas show, however, that no increased output per beat, but only the actual increase of about three times in the pulse rate was needed to account for an increase in metabolism to ten times the basal rate. In none of our subjects was the circulation rate during work increased in even approximately direct proportion to the increase in metabolism until the metabolism had been greatly raised.

Our results differ in certain respects from those obtained by Krogh and Lindhard (15) by the nitrous oxide method. The outstanding difference is that our values for the circulation rate and output per heart-beat during rest are much higher than theirs, and that for the percentage

utilisation of oxygen during rest our values are correspondingly lower. On the other hand our values for the circulation rate and percentage utilisation during work confirm theirs. Their low values for the circulation rate during rest pointed to the conclusion that the general circulation rate increases nearly in proportion to the oxygen consumption.

On the general principle underlying the nitrous oxide method we have no criticisms to make. We wish, however, to point out what seems to us to be a serious source of fallacy in the calculations of Krogh and Lindhard. They take in a maximal breath of a mixture of air, nitrous oxide, and oxygen; then, after a delay of a few seconds, expire about a litre into a Krogh recording spirometer and at the same time take a sample of the alveolar air. After a further delay of 10 to 20 secs. another sample of expired air is taken similarly. From the diminution in the percentage of N_2O in the second sample, and a knowledge, based on the spirometer record and a determination of the subject's vital capacity, of the volume of air which was left in the lungs after the first alveolar sample was taken, the volume of N_2O taken up by the blood during the interval of 10 to 20 secs. is calculated. From this and a knowledge of the co-efficient of absorption of N_2O in blood the rate of blood-flow through the lungs is calculated. At the same time the rate of absorption of oxygen in the lungs is calculated in a similar manner. As, however, they found that the rate of absorption of oxygen as determined in this way was greater, and often far greater, than the rate as determined in the ordinary way just before, or just after, the experiment, they assumed that for some reason the blood-flow through the lungs was temporarily increased during the experiment; and they made a corresponding correction in the calculation of the circulation rate. The correction is a very considerable, though also a very variable one. For the 14 experiments made on Krogh during rest the mean corrected result is 4.06 litres per min., as against 6.42 litres without the correction. Sometimes the correction amounts to 100 p.c.

Now judging from our own experiments, detailed above, as to the impossibility of obtaining an even mixture of oxygen in the alveolar air with a single maximal breath, this correction is not justifiable. The reason why the drop in oxygen percentage between the first and second alveolar sample is so abnormally large seems to us to be that the first alveolar sample gave an oxygen percentage which was higher at the moment than the percentage in the deep alveolar air corresponding to the second sample. It is very probable that the rate of blood-flow through the lungs was abnormally altered to some extent by the experimental

conditions, but it seems much more probable that while the breath was held between the moments of taking the two alveolar samples the flow was diminished than that it was increased. If, however, we take Krogh and Lindhard's results without correction they come much closer to ours, and particularly when allowance is made for the greater weights of our subjects.

It might be expected that an error of imperfect mixture would also affect the N_2O determinations in Krogh and Lindhard's experiments. N_2O , however, is so soluble that it is likely that during the few seconds interval before taking the first alveolar sample the percentage of N_2O in the air of alveolar ducts and-atria would have practically equalised itself with the percentage in the surrounding air sac system of alveoli. Our experiences with CO_2 , which is also very soluble, point in this direction.

The early experiments of Krogh and Lindhard seemed to show an approximate proportionality between circulation rate and general metabolism, and that the percentage utilisation of the arterial oxygen was nearly the same during rest and work. Thus, in experiments which they quote as examples, the calculated percentage utilisation was 60 in Lindhard and 46 in Krogh during rest, while during work of 150 kgm per min it was 73 and 47. In all our subjects the percentage utilisation was much greater during work than during rest, and later experiments by Lindhard (16) show pretty consistently a greater utilisation during work than during rest. In one case he obtained during rest a percentage utilisation as low as 22, which he attributes to the effects of training. None of our subjects showing equally low, or lower, percentage utilisations were at all in training.

The results with the nitrous oxide method give us the impression that, apart from the oxygen correction it is liable to serious error, particularly in the experiments during rest. It seems probable that the rate of blood flow through the lungs may be appreciably diminished during the period of measurement while the breath is held, particularly while the subject is at rest. This would affect the results by the nitrous oxide method, but would not affect the results by our method, since the venous gas pressures, on which our measurements are based, depend on the rate of systemic flow. During work the two methods are in better agreement, and this may be due to the fact that with the greatly increased flow of blood towards the heart the temporary effect on the pulmonary blood-flow of holding the breath is proportionally much less.

In a recent paper in this Journal Liljestr nd and Lindhard (17)

describe experiments in which they compare the results in the same subject with the nitrous oxide method and a modified form of our method which was adopted by Fridericia (18). They conclude that the two methods agree fairly well, and they calculate from their results that their respective circulation rates were 3.9 and 4.3 litres per minute by the first method, and 3.3 and 3.6 by the second. Unfortunately, however, they estimated the arterial CO_2 -pressure by the indirect method of Krogh and Lindhard; and as this method gives, as already mentioned, a lower, and frequently much lower, result than the direct method, which in our opinion is the only reliable one, the comparison seems to us to be vitiated. We are unable to accept as correct the curious form of CO_2 -dissociation curve (drawn from determinations at only three points) which is figured in their paper as applicable to Lindhard's blood.

Fridericia, whilst he made simultaneous determination of the pressures of oxygen and CO_2 , was unaware of the fallacies due to imperfect mixing in the alveoli, so only took one deep breath of his mixture, in which there was about 1.5 p.c. of oxygen. His results were thus rough. On an average, however, they agreed pretty closely with our results of 1914, and indicated that during rest the mean true venous CO_2 -pressure is about 45.5 mm., or 5 mm. above the arterial CO_2 -pressure as found on the only occasions on which it was determined (by the Haldane-Priestley direct method). During light work of 200 kgm. per minute he found that the venous CO_2 -pressure was increased from 46.3 mm. during rest to 52.2 mm.; but there was no determination of the arterial CO_2 -pressure. Fridericia did not use the CO_2 results for calculating the circulation rate, but used the oxygen results in the case of a subject of whose hæmoglobin the dissociation curve had been determined by Krogh. He also compared the results with those obtained on the same subject by the nitrous oxide method. The two methods gave satisfactorily concordant results, and both showed circulation rates and percentage utilisations of oxygen similar on the whole to those in our experiments, but differing considerably from those obtained by Krogh and Lindhard. If we correct for an evident over-estimate of the saturation of the arterial hæmoglobin with oxygen, the percentage utilisations become 21.6 by the nitrous oxide method, and 20.6 by the aerotonometer method, for Fridericia's subject A.

An extensive series of careful experiments by the nitrous oxide method was published by Boothby in 1915 (19). Like those of Krogh and Lindhard, Boothby's experiments give a very low circulation rate during rest, increasing as the metabolism was increased up to

900 c.c. of oxygen per minute. We have, however, already expressed our doubts as to the reliability of the nitrous oxide method during rest. Boothby and Sandiford have also made a series of experiments with a modified form of our own method (20). It seems to us, however, that the time (about 30 seconds) which they allowed to elapse before the second alveolar sample was taken was too long to prevent serious fallacy owing to blood having time to make a complete round of the circulation during the experiment.

It is probable that, owing to the time occupied in expiring the air required for an alveolar sample, the normal alveolar CO_2 -pressure as determined by the Haldane-Priestley method gives results which are slightly too high (probably about 0.5 mm. too high during rest). Krogh and Lindhard drew attention to this error in 1914 (21), but in our opinion enormously exaggerated its importance. This matter is discussed in the forthcoming book already referred to. The actual error may account for the fact that in the experiments on Douglas the circulation rate as calculated from the CO_2 results was on the whole rather greater than the rate calculated from the oxygen results. There may also be slight errors dependent on inaccuracies of our dissociation curves; but it is difficult to see how the combined errors could seriously affect our results. With the same individual and under the same physiological conditions our method seems to give much more consistent results than the nitrous oxide method.

The significance of the greatly increased loss of oxygen and gain in CO_2 of the mixed venous blood during work must now be discussed. There can be no doubt that when muscles are working under normal physiological conditions the flow of blood through them must be enormously increased. During such work as walking fast, running, or working a bicycle ergometer with a heavy load, the general metabolism of the body can be increased 11 times over considerable periods as compared with complete rest fasting. Under these conditions the metabolism in the working muscles must be increased 20 or 30 times. No smaller estimate seems possible. Our knowledge of the gas-contents of the venous blood returning under anything like normal conditions from different tissues is very limited as yet; but Leonard Hill and Nabarro made, in 1895, a very complete series of observations on the percentage utilisation of the oxygen in blood passing through muscles and through the brain in the dog (22). The samples were collected, with precautions against obstruction or abnormality of circulation¹, from the deep femoral vein

¹ Whether these precautions were completely successful in the case of the blood from the deep femoral vein is somewhat doubtful, as Prof. Langley has pointed out to us.

coming from leg muscles and the torcular Herophili in the cranial cavity. It was found that during rest the blood from the muscles had on an average lost 72 p.c. of its oxygen, and the blood from the brain only 20 p.c. During tonic and clonic convulsions produced by absinthe, blood from the muscles had lost 75 p.c., and that from the brain 29 p.c. The contrast as regards utilisation of oxygen in the venous blood from the muscles and that from the brain was thus very great; and assuming that a similar contrast exists in man, we can see why a slight amount of muscular exertion produces such a large effect on the percentage utilisation of the oxygen in mixed venous blood as compared with the resting value. The increase in percentage utilisation during work must also be due almost entirely to the fact that the mixed venous blood during work contains a larger proportion of blood returning from muscles.

Even during rest there must be a certain proportion of highly venous blood returning from muscles, and probably also from the alimentary canal. The blood from superficial veins in man has usually lost about 30 p.c. of its oxygen. Stadie found in superficial arm veins an average of 26.8 p.c. loss (23). As to venous blood returning from various internal organs under normal conditions, there is not much published information; but such data as are afforded by Barcroft's observations (24), and by our own observations on the effects of fasting in causing some lowering of the percentage utilisation of oxygen in the mixed venous blood, suggest that in the digestive organs, though not in the kidneys, the percentage utilisation is higher than in the mixed venous blood. It appears, therefore, that not only must the percentage utilisation in the central nervous system in man during rest be a good deal lower than that for the mixed venous blood, but the blood from the central nervous system must form a quite large proportion of the whole of the blood returning to the heart. In the case of most of our subjects it does not seem at all probable that the blood was losing in the central nervous system more than about 10 or 12 p.c. of its oxygen, or about two volumes of oxygen per 100 c.c. of blood.

The ample supply of vessels leading blood to and from the human brain, and the extremely ample supply to the grey matter, has always impressed anatomists; but probably even this gives an inadequate idea of the relative blood-supply of the grey matter during rest, since the blood-vessels to muscles must be sufficiently large for the blood-supply during great activity. Their blood-supply during great activity must, if Hill and Nabarro's results are approximately representative, be

almost proportionate to the metabolism—i.e. 20 to 30 times what it is during rest.

The low percentage utilisation of oxygen in the brain does not, of course, mean that the metabolism in the brain is low. Probably the metabolism in the grey matter is extremely high as compared with the average for other tissues. What is actually shown is that the brain works at a relatively high pressure of oxygen. This conclusion is confirmed by the extreme sensitiveness of cerebral functions to any diminution in the normal oxygen-pressure of the arterial blood. There may be abundance of dissociable oxygen in the arterial blood going to, and the venous blood coming from, the brain, but if the partial pressure of the oxygen is considerably lowered, the brain becomes more or less paralysed, and after a time irreparably injured.

If the adaptation of the circulation during work were assumed to consist simply in an increase of the blood-supply to the working-muscles, without any diminution in the blood-supply to other parts, calculation shows that the very small increase in general circulation rate in Douglas during light work could not be explained. We must assume that coincidentally with the increased blood-supply to the working muscles there is a diminished blood-supply to other parts. This does not, however, necessarily imply that the oxygen-pressure is diminished and the pH increased in these other parts. It may be that activity in other parts is diminished along with the increase of muscular activity. Moreover the demonstration by Krogh (20) that distribution of blood, and consequently of oxygen, etc., in the tissues, is to a large extent effected by the opening up or closing down of capillary paths seems to show that a tissue may, within limits, remain as well aerated even when the blood coming from it is more venous. By the opening out of more capillary paths the fall in oxygen-pressure between a capillary and the tissue elements which it supplies must be diminished, and in this way the mean oxygen-pressure round the tissue elements may be maintained with a smaller flow of blood. The circulation rate in capillaries during rest is apparently maintained at a higher rate than would be necessary if all the capillary paths were open.

At first sight it may seem strange that with increased blood-flow to the heart, as during muscular work, the heart does not necessarily deliver more blood at each systole. In this connection it must be remembered, however, that, as Bainbridge was the first to show, increased filling of the veins entering the heart produces reflex increase in the frequency of the heart-beats (21). The rate of heart-beat is also affected by the gas-

coming from leg muscles and the torcular Herophili in the cranial cavity. It was found that during rest the blood from the muscles had on an average lost 72 p.c. of its oxygen, and the blood from the brain only 20 p.c. During tonic and clonic convulsions produced by absinthe, blood from the muscles had lost 75 p.c., and that from the brain 29 p.c. The contrast as regards utilisation of oxygen in the venous blood from the muscles and that from the brain was thus very great; and assuming that a similar contrast exists in man, we can see why a slight amount of muscular exertion produces such a large effect on the percentage utilisation of the oxygen in mixed venous blood as compared with the resting value. The increase in percentage utilisation during work must also be due almost entirely to the fact that the mixed venous blood during work contains a larger proportion of blood returning from muscles.

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The ample supply of vessels leading blood to and from the brain, and the extremely ample supply to the grey matter, impressed anatomists; but probably even this gives an impression of the relative blood-supply of the grey matter due to the blood-vessels to muscles must be sufficiently large for activity during great activity. Their blood-supply during great activity Hill and Nabarro's results are approximately

parts, such as the muscular tissues and those of the cerebral cortex. In the latter there is normally a much higher oxygen-pressure and a lower CO_2 -pressure than in the former.

SUMMARY.

1. Details are given and discussed of the method previously described in general terms by Christiansen, Douglas, and Haldane for determining the gas-pressures and gas-contents of the mixed venous blood, and the general circulation rate in man. It is shown that determinations of the oxygen-pressure confirm those of CO_2 -pressure.

2. The effects of oxygenation in raising the CO_2 -pressure of venous blood *in vivo* are the same as those previously observed with defibrinated blood *in vitro*.

3. Figures are given for the venous gas-pressures, circulation rate, blood-output per heart-beat, etc., in several adult men during conditions varying from complete rest fasting to hard muscular exertion. During rest about 5 to 8 litres of blood per minute passed through the lungs in different men, and during the hardest work the estimated flow was about 24 litres per minute. The flow observed during rest was considerably greater than that estimated by the nitrous oxide method.

4. During work there is a great rise in the percentage utilisation of the oxygen in the blood, and owing to this the general circulation rate does not increase in anything like direct proportion to the general metabolism, except when the work has already become so hard that little or no further increase in percentage utilisation occurs.

5. The blood-output per heart-beat is in many subjects no greater during either moderate or hard work than during rest, and is about 120 c.c. for a man of average weight. In other subjects, however, the output per beat is considerably less than this during rest, and increases considerably during work.

6. A moderate excess of CO_2 in the inspired air does not appreciably increase the circulation rate, though it greatly increases the breathing. Excessive removal of CO_2 from the blood by forced breathing diminishes the circulation rate greatly. This helps to prevent excessive fall of pH in the tissues, but also produces anoxæmia.

7. The new facts ascertained are consistent with the view that the rate of blood-flow through different tissues is, as a rule, so regulated that the pressure of oxygen and the pH remain approximately constant round the tissue elements in each tissue. The rise of CO_2 -pressure and fall of oxygen-pressure in the mixed venous blood during muscular

exertion are due mainly to the facts that blood coming from muscles has normally a low oxygen-pressure and high CO_2 -pressure, and that during exertion the mixed venous blood contains a much higher proportion of venous blood from muscles.

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CHEYNE-STOKES RESPIRATION. Part I. Production by adrenalin. By FR. ROBERTS, M.D., M.R.C.P., *Fellow of Clare College, Cambridge.*

(From the Physiological Laboratory, Cambridge)

IN a previous communication⁽¹⁾ I mentioned that in rabbits and cats when a large dose of adrenalin is injected intravenously cessation of respiration followed by a certain degree of periodic respiration is very prone to occur. In the present paper I propose to describe more fully the form of the periodicity and to explain its causation.

When 1 c.c. of 0.02 p.c. adrenalin (Parke, Davis and Co.) is administered to a rabbit which has been anaesthetised with urethane and C.E. mixture the characteristic form of a well-developed Cheyne-Stokes respiration may be thus described. Respiration rapidly decreases in depth and comes to a complete stop. This period of apnoea lasts from 15-30 seconds and is occasionally interrupted in the middle by a single respiration which is so suggestive of the isolated respiratory movements found in certain stages of asphyxia as to confirm one in the belief that during this apnoeic period the centre is suffering from oxygen want. After the apnoea, respiration, at first very shallow, recommences at the previous or at a different rate. It increases progressively in depth, attains a maximum and then decreases progressively until it very nearly disappears. This group of respirations lasts about 30 seconds and in the tracing is exhibited as a regular spindle, the rate of the respiratory movements being perfectly regular. Then follows a period of shallow breathing in which the movements are only just visible on the tracing and on inspection of the abdomen. This period has about the same duration as the first period of apnoea. A second group of respirations then occurs usually developing more gradually than the first. This may be followed by another period of shallow breathing (though less shallow than the last) which leads to a period of steady respiration. Throughout the period of high blood-pressure the respiration is subnormal in depth, recovering as the blood-pressure falls. The rhythm of these respiratory waves is from 30-80 seconds.

Frequently, however, periodicity does not occur. There is an initial arrest or decrease of respiration which is followed by an almost regular

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recovery as in Fig. 6. Sometimes one group of respirations is seen followed after shallow respiration by regular recovery. More rarely Cheyne-Stokes respiration of a more prolonged and pronounced kind is obtained. An example of this has already been given(1). I have been unable to discover any condition which favours the production of the phenomenon. It seems to have nothing to do with age or sex and appears to be entirely a matter of individual susceptibility. While it may be stated that it is more easily produced the larger the dose this is true only up to a certain point, for animals which fail to show it with 0.01 p.c. adrenalin still fail to give it with a dose ten times as strong. It may further be remarked that the tendency to periodicity like the tendency to arrest becomes less marked on repeated injection. In general, the tendency to Cheyne-Stokes respiration varies directly with the initial effect of adrenalin upon the depth of respiration. Finally, it occurs much more frequently in the rabbit than in the cat.

Sometimes minor waves of respiration having a periodicity of 5-15 seconds are observed. They may be superimposed upon the larger waves as in Fig. 5. An interesting point shown by this figure is that the periodicity of the minor waves becomes longer during the shallow period of the major waves. An instance of the combination of major and minor waves in the cat has already been given(1).

In my previous paper I gave reasons for believing that the arrest of respiration was due to asphyxia of the respiratory centre owing to the vaso-constrictor effect of adrenalin. This view, that the proper functioning of the centre is dependent upon a certain amount of oxygen does not run counter to the generally accepted belief that the activity of the centre varies directly with the hydrogen-ion concentration of the blood bathing it. We are here well outside the range within which the delicate action of the acidity of the blood comes into play. In this opinion we are substantially in agreement with the view expressed by Leonard Hill that "a certain blood-pressure is necessary to provoke respiration—that is to say, a certain amount of blood must flow through the centre(2)."

If therefore we are correct in ascribing the arrest of respiration to vaso-constriction it is natural to enquire whether the Cheyne-Stokes respiration is also vascular in origin. There are certain *a priori* reasons for believing this to be the case: first, there is the fact already mentioned that those individual animals which show a marked arrest exhibit periodicity; secondly, there is the fact that periodicity is most easily produced in rabbits—animals in which the vaso-motor system is notoriously readily disturbed. In other words we should expect to find rhythmic variation

in the calibre of the cerebral vessels corresponding to the rhythmic changes in the depth of respiration, the vessels being shut completely or nearly completely during apnoea and open during the groups of respiration. At the same time it is hardly to be expected that such changes in the cerebral vessels would be shown in a tracing of the general arterial pressure since the effect might be masked by other factors, as for instance changes in the calibre of blood vessels in other parts of the body, changes not synchronous with those undergone by the cerebral vessels.

In order therefore to find out what is happening in the vessels within the skull I have taken records, simultaneously with those of general arterial pressure, of the pressure in the circle of Willis, using the device first adopted some years ago by Corin, in Friederiq's laboratory and afterwards developed by Hurthle and later still, Cavazzani, for the purpose of investigating the existence of vaso motor fibres to the brain.

In the rabbit, according to Krause(3), the internal carotid artery gives off no branches until it enters the skull. Within the skull it gives off two branches, the superior ophthalmic and the posterior communicating. It then divides into anterior and middle cerebral, the former of which is large, the latter small. By the posterior communicating the internal carotid is connected with the posterior cerebral and therefore enters into the formation of the circle of Willis. The anterior communicating is absent in this animal—the circle therefore is not complete.

The experimental procedure is as follows. The rabbit having been anaesthetised with urethane and C F mixture and the trachea tube inserted, the common carotid is dissected out and its superior thyroid branch ligatured. The bifurcation into internal and external carotids is then cleared of the nerve filaments which surround it and the external carotid is ligatured at its origin. A cannula is then tied into the upper part of the common carotid, its nozzle pointing towards the brain. On connecting this cannula with a mercury manometer a record is obtained of the blood-pressure at the point where the internal carotid

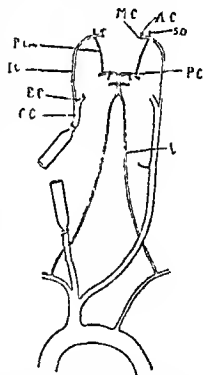


Fig. 1 Showing blood supply of the brain in the rabbit (after Krause), and arrangement of cannulae.

M C, Middle Cerebral,
A C, Anterior Cerebral,
S O, Superior Ophthalmic,
P C, Posterior Communicating,
I C, Internal Carotid,
E C, External Carotid,
C C, Common Carotid,
V, Vertebral,
P C, Posterior Cerebral.

opens into the circle. A diagram of the arteries is shown in Fig. 1. General arterial pressure is recorded by inserting a cannula in a downward direction into the lower part of the common carotid of the same side. Respiration is recorded by connecting one limb of the trachea tube with a tambour.

By the time the blood has passed through the circle into the internal carotid, pulsation due to the heart-beat has been considerably damped off by the resistance of the narrowest vessel, which is probably the posterior communicating. Distinct pulsation can be seen at the nozzle of the upper cannula at the point where the blood joins the sodium

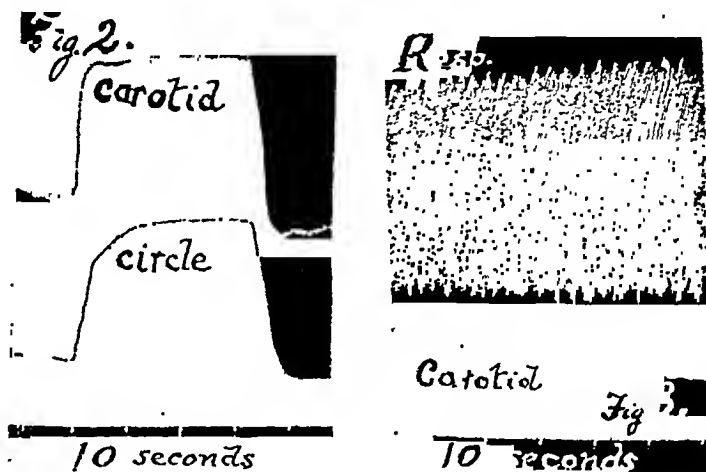


Fig. 2. Showing response of the circle of Willis to changes in the aortic pressure produced by clamping and releasing the abdominal aorta.

Fig. 3. The same rabbit as in Fig. 5. Minor waves during recovery from a dose of adrenalin. Note oscillations in blood-pressure.

sulphate solution with which the cannula is filled. Pulsation is indeed visible at the surface of the mercury in the manometer but it is too feeble to show itself in the tracing.

The pressure in the circle as shown by this method is surprisingly high. It is seen in Figs. 4 and 5 to be very little less than the aortic pressure. Sometimes there is a difference of about 20 mm. between them. After an injection of adrenalin the circle pressure may be as high as 160 mm. or more.

Before any conclusions can be drawn from the record of circle pressure, it is necessary to determine the readiness with which this pressure responds passively to changes in the aortic pressure. This is

best done by subjecting the latter to sudden changes by alternately closing and opening the abdominal aorta above the origin of the cœliac axis artery. A typical example is seen in Fig. 2. The change in pressure in the circle follows immediately upon the change in the aortic pressure and is nearly as sudden. In some individuals, however, the circle responds very slowly. This must undoubtedly be due to unusual narrowness of some of its vessels. In such cases, the tracing is not a faithful record of cerebral pressure as regards time-relations. I have therefore discarded those cases in which a lag exists and followed out those only in which the circle tracing follows promptly upon changes in the aortic pressure. In the cat considerable lag always occurs owing to the smallness of the internal carotid. The method cannot therefore be employed in this animal.

The effect of an injection of adrenalin can be followed from Fig. 4. The carotid pressure rises suddenly and after touching 170 mm. has an

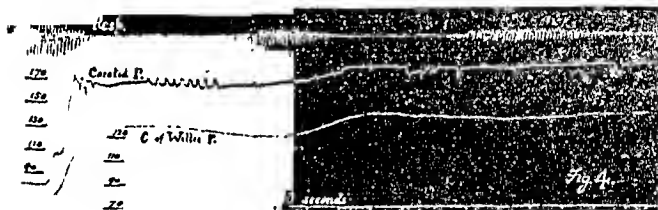


Fig. 4. Rabbit. Adrenalin, 1 c.c. 0.02 p.c. Description in text.

average pressure of 164 during the beginning of the first period of apnœa. At the same time, the pressure in the circle of Willis rises more slowly and reaches 140. Towards the end of the apnœa the carotid pressure falls slightly—to 160. At the same time the circle pressure undergoes a more pronounced fall. As respiration is resumed the carotid pressure is at first level and then begins to rise slightly but the circle pressure continues to fall to 131 which it reaches as respiration attains its maximum development. Here the circle pressure begins to rise while the carotid pressure also rises but less rapidly. Both attain their summit at the beginning of the second apnœa, the carotid being 175, and the circle 150. Towards the end of the apnœa carotid pressure begins to fall, from 175 to 172, the circle pressure falling simultaneously from 150 to 144. During the fall respiration re-commences and when the circle pressure is at its lowest respiratory movements are at their height. As respiration diminishes again both pressures rise.

It will therefore be seen that the rhythm of respiration corresponds with fluctuations in pressure in such a way that respiration begins when the pressure is falling and stops when it is rising. It will further be seen that the changes in cerebral pressure are much greater than those shown in the carotid. During the first apnoea the circle falls 9 mm. while the carotid falls 4. Then the circle rises 19 while the carotid rises 15. After that the circle falls 6 while the carotid falls 3.

If the changes in the cerebral pressure were produced passively by changes in general pressure or if they were due to alterations in the calibre of arterioles undergone in all parts of the body simultaneously they would be less marked than the changes in the carotid pressure, first

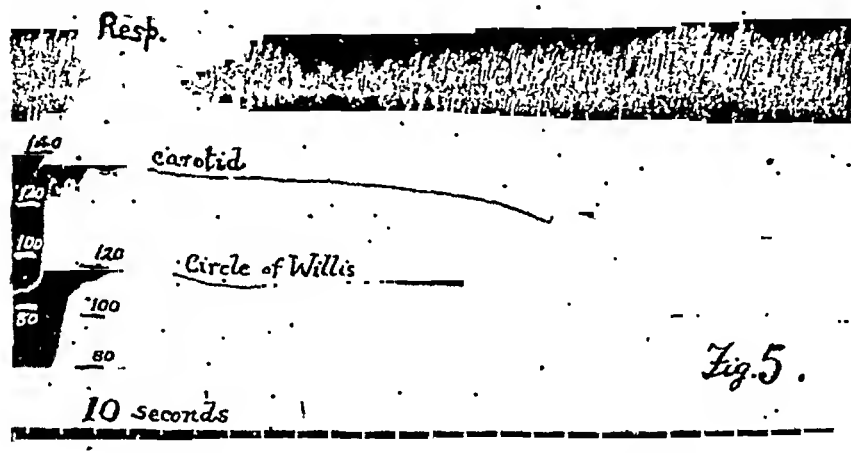


Fig. 5. Rabbit. Adrenalin, 1 c.c. 0.02 p.c. Several minor waves superimposed on one major wave.

because the pressure in the circle is less than that in the carotid and secondly because the blood encounters a certain resistance in flowing through the circle. It is clear therefore that the fluctuations in cerebral pressure are too great to be merely the effect of fluctuations in the aortic pressure.

An unusual feature of the tracing in Fig. 4 is that the blood-pressure is higher during the second apnoea than it was during the first. As a rule the pressure after attaining its maximum at the beginning shows a general downward trend as shown in Fig. 5. This figure shows one major wave of respiration which corresponds to a marked trough in the circle pressure while the general pressure is falling steadily.

The explanation of the major waves of Cheyne-Stokes respiration is

therefore quite simple. During the first period of apnœa the arterioles of the brain with those of the rest of the body are constricted. The cerebral vessels then relax, this being shown by the fall in circle pressure. Sufficient blood is therefore admitted to the respiratory centre to allow it to function. The second rise in circle pressure must mean that the arterioles are closing again, the closure corresponding to the second period of apnœa and so on. In other words adrenalin causes Cheyne-Stokes respiration because it causes rhythmic changes in the calibre of the cerebral vessels. In all the experiments which I have performed this relation between cerebral pressure and respiration has been obtained.



Fig 6 Rabbit. Adrenalin, 1 c.c. 0.01 p.c. Almost regular recovery of respiration except for a very slight notch at A corresponding to a tendency to a secondary elevation in circle pressure.

In those cases where respiration recovers uniformly without periodicity the waves on the circle pressure are absent. This is shown in Fig. 6.

Previous observers have found evidence of rhythmic contraction of blood vessels. Cow(4), using ring preparations of surviving arteries occasionally found it. The sheep's gastric artery on a dose of adrenalin following two doses of ergot showed such a rhythm, each complete wave lasting about 25 seconds. Rhythmic contraction of surviving arteries has been found by several other observers. Recently McDowall(5), perfusing the pulmonary circulation of young rabbits and cats, found waves at the rate of 1 to 10 per minute. They were stimulated by adrenalin and by pituitary extract and they gave evidence of complete

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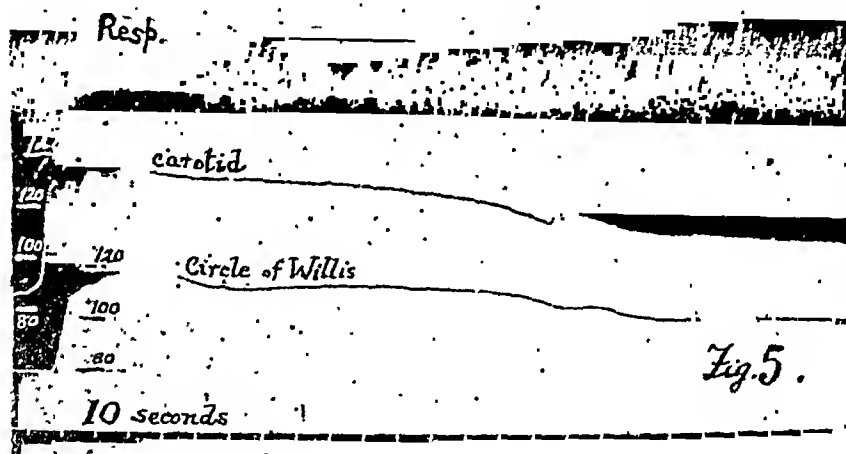


Fig. 5. Rabbit. Adrenalin, 1 c.c. 0.02 p.c. Several minor waves superimposed on one major wave.

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The explanation of the major waves of Cheyne-Stokes respiration is

2. Considerable differences exist between individual animals in their tendency to Cheyne-Stokes respiration.

3. The major waves are due to rhythmic changes in the calibre of the cerebral vessels, the respiratory centre being quiescent owing to oxygen want during closure of the vessels and active when the vessels are open.

4. The minor waves seem to be associated with small oscillations in general blood-pressure.

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deceitful no anæsthetic was given. The results were the same as in anæsthetised cats.

Whilst the conditions given by Dieden are not necessary in order to obtain a secretion on local injection of adrenaline, nerve section favours secretion. Section of the brachial or sciatic nerves causes increased blood flow and rise of temperature in the foot. The degree to which this is produced varies greatly in different conditions. In my experiments, though section of the nerves nearly always caused flushing of the foot, the flushing was soon over, and on injecting Ringer's fluid or adrenaline into the pad of the foot on each side, there was not infrequently no difference in the secretion on the two sides, sometimes both secreted practically equally, occasionally there was no secretion on either side. So far as the experiments went, the effect of nerve section was greater when Ringer's fluid, than when adrenaline was injected. I did not in any experiment obtain no secretion on the intact side when there was a moderate secretion on the side with the nerves cut; this, however, would, I think, inevitably occur in a certain state of gland excitability if the foot with nerves cut were flushed and warm and that on the intact side were pale and cool—a condition which is common for two or three weeks when the animals are kept alive after nerve section.

The excitability of the sweat glands varies greatly in different cats. This is shown by the very different amount of secretion obtained on injecting a small amount of pilocarpine. The stimulus set up by injecting fluid is a weak stimulus compared to that set up by pilocarpine. In consequence, Ringer's fluid or adrenaline solution sometimes causes no secretion, sometimes a slight or sometimes a free secretion.

The results obtained above are *prima facie* accounted for by variations in gland excitability and variations in blood supply, but as Dieden had considered that he obtained some slowing of the secretion caused by pilocarpine by stimulation of the posterior roots of the lumbo-sacral nerves, it was necessary to observe the effect of such stimulation. I made seven experiments on this point. The posterior roots of the 6th and 7th lumbar nerves were cut and the peripheral ends stimulated after a small amount of pilocarpine (0.1 c.c. of 0.01–0.1 p.e. of the nitrate) had been injected into the pad of the foot. In none was there any decrease of the secretion. On watching with a lens, the steady increase in the size of the drops could be seen. Instead of a decrease there was usually a slight increase in rate of secretion. The increase was obviously due to increased circulation caused by the stimuli. The foot in each experiment flushed, sometimes intensely—an antidromic action I deal

and then successively 2, 1, and 1 mgm. pilocarpine. No secretion in either hind foot, nor on stimulating the sciatic nerves. The fore feet were not noticed. Most of the sweat glands on the injected side had a wide lumen except near the surface.

(c) Decerebrated. One sciatic nerve cut; into the pad of this side Ringer's fluid was injected. Into the other pads, 1 p.c. BaCl_2 , Ringer's fluid, Ringer's fluid and then amy nitrite, were respectively injected. 5 mgm. of pilocarpine injected into the jugular vein 1½ hours after the beginning of the experiment. Left post.-tibial nerve stimulated. No secretion at any time. Fæces in rectum white.

I may note that two of these animals were long-haired, and the third had unusually long hair between the toes, suggesting a connexion between slight excitability and breed of cat, but in the one subsequent experiment on a long-haired cat, the glands secreted fairly freely.

When the amount of pilocarpine is lessened to a certain degree, or when the gland excitability is slight, either fore or hind feet may secrete most. There may also be unequal secretion in the two feet on the same side, and in different parts of any one foot; in general the centre of the mid pad secretes more freely than any other part. There may even be differences in the inner and outer halves of any one toe. Similarly, on injecting Ringer's fluid into the pads of the two fore feet, or into those of the two hind feet, the amount of secretion is not always the same.

It is well known that secretion is less in old than in young cats. The less response in old cats is probably due in the first place to obstruction of the outflow by increase of the horny layer, and then to injury of the glands caused by retention of secretion. Another cause of difference in response is a difference of temperature of the feet; cold, as is known, decreases greatly the secretory activity. According to Straus(7) cooling a patch of skin nearly to freezing point in man not only prevents secretion being obtained on local injection of pilocarpine, but has a prolonged after effect.

When pilocarpine has been given in sufficient amount to cause a fairly free secretion, a second dose given when the secretion has nearly stopped has sometimes a slight effect only. Prolonged anæsthesia with chloroform and ether, loss of blood, such as sometimes occurs in decerebration, also greatly reduces secretory activity. In all these conditions there is a fall of blood-pressure. Apparently continued secretory activity requires a good circulation.

On local injection of pilocarpine into a small area of the secretory surface of one foot, the extent of the outspread of secretion depends partly upon the concentration of the pilocarpine and partly on the excitability of the glands. Early in an experiment 0.1 c.c. of 1 p.c. pilocarpine injected into the pad of one foot is, as I have said, not infrequently sufficient to cause a free secretion on the pad and toes of

all the feet, the secretion may, however, be confined to the injected foot, in which case it begins in the injected mid pad, spreads first to the side pads, and then to the toes. With sufficient reduction in the concentration of the pilocarpine, the secretion is confined to the injected region, as it is when Ringer's fluid or adrenaline is injected. When pilocarpine causes a slight secretion only, the other fluids cause none. The level of the injection into the subcutaneous fat tissue probably influences the result and with the same level of needle insertion, the level of the fluid injected is somewhat different according as the opening of the needle faces the inner or outer surface.

Bearing these points in mind, we may consider the results of denervation. Six experiments were made. In each case 0.1 to 0.15 p.c. of the solution was injected into the middle of the pad from the front. In two experiments pilocarpine was injected first into the undenervated pad. In two it was injected first into the denervated pad. In one, Ringer's fluid was injected and this was followed by adrenaline. In one Ringer's fluid was injected followed by pilocarpine. A summary of the results of the first four experiments is given in Table I.

TABLE I. Effect of pilocarpine after section of the sciatic nerve

Nerve cut	Time allowed for degeneration	Place of injection of pilocarpine	Amount of secretion in denervated foot
1 Left sciatic	13 days	Right hind foot Denervated foot	Very slight Slightly increased
2 Left sciatic	14 days	Right hind foot Denervated foot	A few very small drops Slightly increased
3 Right sciatic	15 days	Denervated foot	Slow but good
4 Right sciatic	38 days	Denervated foot	Copious

In Exps 3 and 4 the secretion caused by pilocarpine was on the whole secretory surface of the foot. In Exps 1 and 2 it was irregular on the toes. In the cut nerves no sound fibres were found. In Exp 4 the fibres were band fibres.

Exp 1. The secretion in the undenervated feet was sub normal. Two of the toes on the denervated side were somewhat swollen at the base. Sections of the pads of the hind feet showed under the microscope a marked difference on the two sides. On the denervated side the horny layer was not continuous, the Malpighian layer was thinner and the glands of smaller diameter.

Exp 2. The epidermis on the denervated side was scaling off. There was a fairly copious secretion on the undenervated hind foot but of less duration than usual and a sub normal secretion on the fore feet.

Exp 3. Ringer's fluid caused a fairly free secretion in the pad of the right fore foot. The secretion in the undenervated hind foot began more slowly than in the denervated but at its maximum was better and lasted longer.

Exp 4. Some details of this will serve to illustrate the method, and some of the facts mentioned in the earlier part of this paper.

Right sciatic nerve cut 38 days. Chloroform. Tracheotomy, C.E. mixture. The injections were of 0.1 c.c. into the mid pad.

		Fore feet		Hind feet	
		Right	Left	Right	Left
		Inject			
10.20-10.25	Ringer's fluid	0.1 p.c. adrenaline		—	30 p.c. alcohol
10.30	Fairly good secretion on mid pad	Trace of secretion on mid pad		Dry	Trace of secretion on mid pad
10.35	Nearly dry	Dry		Dry	Dry
				Inject	
				1 p.c. pilocarpine	
10.45	Very slight secretion on mid pad, copious secretion on side pads and toes	Slight secretion on inner and outer toes, none on rest of foot		Copious secretion on pad and toes, lasts longer than on left	Very slight secretion mid pad, copious on side pads and toes

Subsequent injection of pilocarpine into the left mid pad caused a good secretion in it.

Mid pad = the large middle part of the pad. Side pad = the small eminences at the sides of the mid pad.

The point of most importance in these experiments is that the secretion in the denervated foot in Exps. 3 and 4 was on the whole of the secretory surface. Ringer's fluid—and adrenaline—only cause secretion in the injected area. Consequently the secretion produced was mainly due to pilocarpine and not to the fluid in which it was dissolved.

Exps. 1 and 2 approximate to those of earlier observers. The secretion in the denervated foot caused by injecting pilocarpine elsewhere than in the foot itself was so slight that close examination with a lens was required to be certain there was any. Further, although the secretion in the other feet was less than usual, the contrast between the secretion on the three undenervated feet and that on the denervated foot was very marked. There appears, then, to be a considerable difference in the degree of decrease of excitability consequent on denervation in different cats. How far the apparent difference is real is difficult to say with certainty. The production of secretion by a low concentration of pilocarpine depends on the original excitability. A given decrease of excitability caused by denervation will allow a secretion to be produced by a low concentration of pilocarpine if the original excitability was high, and prevent it being produced if the original excitability was low. The weakening of the heart beat and decrease in circulation rate caused by pilocarpine varies in different animals; if, as is probable, the secretory activity varies with the circulation rate, injection of pilocarpine elsewhere than in the denervated foot will tend to a variable extent to

decrease the response of the glands to pilocarpine subsequently injected locally. In Exp. 1 the second injection of pilocarpine caused but slight secretion in the undenervated foot. Lastly, there is the variable change in the skin caused by denervation. Whilst both the animals of Exps. 1 and 2 were apparently perfectly healthy, and there was no abrasion of skin in any part of the foot, microscopic examination showed that there was a pathological change in the epithelium in Exp. 1 and the horny layer had formed scales in Exp. 2. Whilst it may be questioned whether in these experiments a free secretion would not have been obtained if the denervated foot had been warmed and 1 p.c. pilocarpine injected at once into the pad, the results as they stand are best accounted for on the theory that the decrease of excitability varies in different cats.

Horsley's observations on man tend to show that the decrease in excitability caused by nerve section is due to disuse, for compression of the spinal cord would not cause degeneration of the post-ganglionic fibres. Thus the change which occurs in the sweat glands may be regarded as a feeble form of the change which occurs in the salivary glands on severance of their pre-ganglionic fibres. Whether there is a greater change on cutting post-ganglionic fibres does not on the existing evidence seem likely.

In the two remaining experiments the posterior tibial nerve was cut. This contains all or nearly all the secretory fibres for the foot. At the end of the experiment the peroneal nerve was stimulated. In Exp. 5 it caused no secretion; in Exp. 6 it was difficult to be certain of entire absence of effect since a slow secretion was going on from the pilocarpine which had been injected, but if it had any effect it was only trivial.

Exp. 5. Right posterior tibial nerve cut 12 days; 0.1 c.c. injected into the mid pad.

Ringer's fluid injected into each foot—a trifling secretion in the left fore foot only.

Adrenaline .1 p.c. injected except into the left fore foot—no secretion except possibly a speck or two in the left hind foot.

Pilocarpine 1 p.c. injected into the right fore foot—slow secretion in this foot; slight secretion in the pad of the left fore foot; possibly a trace in the hind feet.

Pilocarpine 1 p.c. injected into the right (denervated) hind foot—slight secretion on the pad and one toe of this foot, and rather more on the opposite side. By this time there was a fairly good secretion on the right fore foot, and a moderate secretion on the left fore foot. Subsequent injection of 4 mgm. of pilocarpine did not alter the result.

This experiment showed that denervation did not increase the response of the glands to Ringer's fluid or to adrenaline. The result of injecting Ringer's fluid into the undenervated feet indicates that the excitability was sub-normal. The effect of pilocarpine was reduced by the previous injections, but as pilocarpine injected into the denervated foot caused

somewhat more secretion on the opposite side, the excitability of the glands must have been reduced by denervation.

Exp. 6. Right posterior tibial nerve cut 14 days.

Ringer's fluid injected into both hind feet and the left fore foot—good secretion in all, the secretion decreased first in the denervated foot.

Adrenaline 0.1 p.c. injected into the right fore foot—slight secretion ceasing in a few minutes.

Pilocarpine 1 p.c. injected into the pad of the right (denervated) foot—gradually a copious secretion on the whole foot, somewhat less secretion in the left hind and left fore feet; slight secretion in the mid pad of the adrenaline injected foot, fairly good secretion in the rest of the foot but less in the 2nd and 3rd toes than in the 1st and 4th. Later the secretion in the denervated foot was less than in the left hind and left fore feet, and injection of pilocarpine into the undenervated hind foot caused copious secretion in this foot and comparatively slight increase in the others.

In this experiment the excitability in the undenervated feet was high. Ringer's fluid caused nearly as much secretion in the denervated as in the undenervated foot. Pilocarpine had a similar effect, but the secretion was much greater and lasted much longer. With equal pilocarpine stimulation the secretion was less on the denervated than on the undenervated side.

On local injection, pilocarpine 1 p.c. is a very much stronger stimulus than Ringer's fluid. We have seen that denervation does not increase the response to Ringer's fluid. Consequently, if in a denervated foot pilocarpine causes secretion, it must in part at least be due to pilocarpine itself. The secretion, then, obtained by Anderson and myself six weeks after denervation was in part at least due to pilocarpine.

CONCLUSIONS.

Ringer's fluid injected into the pad of a cat's foot commonly causes secretion of sweat and usually causes more than does adrenaline solution. The probability is that adrenaline only causes secretion in virtue of the fluid in which it is dissolved. Secretion is obtained whether the nerves are cut or not. With both fluids, nerve section favours secretion in consequence of increase of circulation in the foot, but in ordinary experimental conditions the increase of circulation as judged by the colour of the foot, is often slight. Stimulation of the peripheral ends of the posterior roots of the 6th and 7th lumbar nerves causes no secretion: if a secretion is going on, the stimulation causes a slight increase in consequence of the increased circulation which it produces. Within certain limits of concentration of adrenaline and pilocarpine there is a mutual antagonism in their action on secretion.

Ringer's fluid and adrenaline, when they cause secretion, cause it in the injected area only; pilocarpine as is known, unless in minimal quantity, has a widespread effect. Thus on injecting pilocarpine into a denervated foot, the action of pilocarpine can be distinguished by the secretion it causes outside the injected area from that of the fluid in which it is dissolved, unless the excitability of the glands is slight. If the excitability is slight, the action can still be distinguished since Ringer's fluid will then cause no secretion.

Denervation always causes some decrease of response to pilocarpine, but the decrease may be slight. A copious secretion may sometimes be caused by pilocarpine, injected in the gland region, even 38 days after nerve section. The secretion is not confined to the injected area as it is when Ringer's fluid is injected, and I conclude, as formerly, that pilocarpine acts directly on the gland cells—probably on a receptive substance in them.

In some cases pilocarpine gives rise to very slight secretion after denervation. Contributory causes to this state are, original slight excitability, insufficient concentration of pilocarpine, and pathological changes in the skin. There seems, however, to be a loss, variable in degree, due to disuse.

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NOTE. Burn in recent unpublished experiments finds that the absence of secretion in response to pilocarpine injected elsewhere than the foot is due to defective circulation.

RENAL BLOOD-FLOW AND GLOMERULAR FILTRATION. BY E. B. MAYRS AND J. M. WATT.

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RECENT measurements of the blood supply to the kidneys have shown that it is much more abundant than was formerly supposed. None of the earlier estimates by such observers as Heidenhain(1), Landergren and Tigerstedt(2) appear to have exceeded 0.5 c.c. of blood per gram of kidney substance per minute, while the later work of Tribe and Barcroft(3), and of Barcroft and Brodie(4) places the blood-flow at four times this amount. The application of these and similar results to the human kidney is of interest in providing us with a means of ascertaining the efficiency of this organ as an excretory apparatus. If the blood-flow through the kidneys in man is taken as 2500 litres in 24 hours¹, and the average urea content of the blood as 0.2 gm. per litre, the total amount of urea which passes through the renal vessels each day is 500 gm. But the daily excretion of urea is only about 30 gm., or 6 p.c. of the quantity supplied to the kidney by the blood stream. Remembering, however, that the blood urea is distributed equally between plasma and corpuscles, and that the time spent by each unit of blood in the renal circulation is insufficient to allow an appreciable amount of diffusion to occur, it is evident that the relative proportions of plasma and corpuscles must be considered when determining how much urea is actually available for excretion. If we assume that half the total volume of blood consists of plasma it follows that 12 p.c. of the available supply of urea is eliminated. This means that 88 p.c. of the plasma has not come into effectual contact with the renal cells, and is altered in its passage through the kidney only in so far as it is diluted with the fluid that has come into such contact.

In order to verify this conclusion it is necessary to investigate the above relationship in animals, since the amount of blood passing through the human kidneys can only be inferred from a general analogy, which may lead to incorrect deductions for any particular period. We had attempted, in rabbits, to correlate the renal blood-flow and plasma urea content with the output of urea by the kidneys, and obtained results which did not differ greatly from the conjectured value. We were soon

¹ This figure is obtained by assuming that half the blood in the body can pass through the kidneys in one minute—a blood-flow which appears usual in rabbits.

convinced, however, that urea was not the most suitable urinary constituent to employ for our measurements, since, as one of us has shown (5), it is less efficiently eliminated by the kidney than sulphates, phosphates, and creatinine. Urea is probably not a useless waste product, as is generally supposed, but a substance which is retained in the body until its concentration reaches some low threshold value, at which the excess begins to appear in the urine. Sulphate was therefore chosen in our subsequent experiments as evidence of the proportion of plasma which comes into efficient contact with the renal cells.

Accepting the view that secretion of urine depends on filtration through the glomerular capsule and selective reabsorption in the tubules (6), efficient contact occurs during filtration and reabsorption; and our purpose was to ascertain what fraction of the plasma was actually subjected to this process, or, in other words, to measure the amount of glomerular filtrate in terms of the total plasma circulating through the kidney. To do this, it is necessary to know (a) the volume of plasma which circulates through the kidney in a given time; (b) the total amount of sulphate contained in this plasma, and its concentration; and (c) the quantity of sulphate which escapes in the urine during the same period. We suppose that filtration through Bowman's capsule does not alter the concentration of sulphate, and that all the sulphate which is filtered appears in the urine. Hence

$$\frac{\text{Glomerular filtrate}}{\text{Total amount of plasma in kidney}} = \frac{\text{Sulphate excreted}}{\text{Sulphate in plasma in kidney}}.$$

Method. After cannulae had been introduced into the trachea, carotid artery, and jugular vein, the abdomen was opened and arrangements were made for measuring the blood-flow through the left kidney by the use of Condon's pneumatic clamps, with a cannula of known volume in the inferior vena cava; the method recently described by Cushny and Lambie (7) being closely followed. A capillary cannula was inserted into the left ureter. A solution was injected intravenously which contained 10 p.e. of anhydrous Na_2SO_4 and usually 6-7 p.e. of gum arabic. The amount given was about 20 c.c. When the injection was complete, urine was collected for a definite time (generally about ten minutes), several blood-flow determinations being made at intervals during this period. A carotid pressure record was kept throughout the experiment. The volume of blood which passed through the kidney during the collection of urine was calculated from the average blood-flow, and the quantity of plasma represented by this volume was ascertained by means of the hæmatoerite. Sulphate was estimated in plasma and urine either gravimetrically or by the benzidine method.

Results. The figures obtained in a series of experiments are shown in the accompanying table.

No	Weight of rabbit, grms.	Injection, c.c.	Average blood-pressure, mm. Hg.	Average blood-flow, c.c. per min.	Vol. of urine, c.c. per min.	Hæmato-crite reading Corpus-cles %	% Na_2SO_4 in plasma	Na_2SO_4 excreted, grms per min.	Glomer-ular filtrate c.c. per min.	% fluid loss from plasma
1.	1800	20	77.0	28.2	1.95	13.0	.3490	.0202	5.80	23.6
2.	1700	20	62.2	18.0	0.73	22.5	.5153	.0149	2.89	20.7
3.	3200	20	61.5	50.0	0.64	27.5	.2773	.0160	5.78	16.0
4.	2080	20	73.0	22.0	—	17.0	.6085	.0071	1.17	6.4
5.	1800	20	77.0	21.5	1.50	26.0	.5805	.0260	4.47	28.1
6.	1850	20	62.0	7.8	0.20	19.0	.7036	.0047	0.67	10.5
7.	1750	11	64.0	36.0	0.67	25.0	.3877	.0069	1.78	6.6
8.	2400	18	81.0	11.0	0.47	32.5	.3513	.0078	2.22	29.9
9.	2100	n.g. 20	71.0	13.6	0.51	28.5	.7537	.0109	1.45	14.9
10.	1720	n.g. 10	66.0	35.3	2.20	30.0	.3471	.0324	9.33	37.7
11.	2700	n.g. 20	80.0	28.7	0.49	27.0	.2142	.0077	3.59	17.2

An example may be given to show how the figures of the last two columns were obtained: In Exp. 1 the blood-flow through one kidney per minute was 28.2 c.c., of which 87 p.c. or 24.5 c.c. was plasma. This contained 0.349 p.c. sulphate, so that the total sulphate passing through one kidney per minute was 0.0855. The sulphate in the urine per min. was 0.0202, so that only 23.6 p.c. of that circulating through the kidney was eliminated. Assuming that all the sulphate in the glomerular filtrate appeared in the urine, the filtrate must have been 23.6 p.c. of the 24.5 c.c. of plasma circulating through the kidney, or 5.8 c.c. per minute.

In the first seven experiments gum solution was given for the purpose of retaining as far as possible the normal physical character of the blood. In the remaining four cases gum was omitted from the injected fluid in order that the effect of colloid dilution on the work of the kidney might be observed; and the hæmatocrite readings for these experiments can probably be taken as roughly indicating the relative colloid osmotic pressures which existed in the plasma. Increased blood-pressure and acceleration of the renal blood-stream are results of the intravenous injection of sulphate, but it is doubtful whether these changes do more than compensate for alterations in the opposite direction caused by anæsthesia and operative shock.

The blood-flow through the kidney is evidently subject to great variation. In Exp. 3, where the average renal circulation in an unusually large rabbit is given as 50 c.c. per minute, the maximum value observed was about 70 c.c. per minute, or 140 c.c. for the two kidneys. This cannot be less than half the total volume of blood in the vascular system, and may be a good deal more. Other experiments gave results as low as 7 or 8 c.c. per kidney per minute, but in these cases the viscosity of the blood appeared to be so great as to render its passage through the capillaries a matter of difficulty. We were satisfied, however, that considerable changes in the renal blood supply must occur under normal conditions,

and believe that the calibre of the renal arterioles is subject to frequent variation

The amount of glomerular filtrate and the proportion it bears to the total plasma circulating through the kidney also present no obvious relation to any of the factors recorded. The amount varied from 9.33 to 0.67 c.c. per minute, and the ratio between it and the total plasma from 37.7 to 6.4 p.c. in different experiments.

The two factors on which the calculation of the filtrate is based are the amounts of sulphate in the plasma and in the urine respectively, and it is therefore necessary to consider how far these vary together and how far the excretion of sulphate is determined by the changes observed in our experiments. In the first seven experiments in which the colloids of the blood were approximately normal, there seems to be no parallelism between the sulphate of the plasma and the amount excreted. The two experiments (Nos. 4 and 6) in which the plasma content was highest, show the lowest elimination, while in Nos. 1 and 3, in which the plasma contained least, the sulphate of the urine was much higher, but in No. 7, in which the plasma content is low, the sulphate excreted is also low. The experiments in which no gum was injected similarly show no correspondence between the content of the plasma and of the urine in sulphate. There can be no question that the concentration of sulphate in the plasma does not determine immediately the amount eliminated by the kidney, and the view that the presence of such a substance in the blood stimulated the renal cells to excrete it is incompatible with our observations as well as with those of previous observers.

Similarly no immediate connection can be drawn between the amount of sulphate in the urine and the blood-pressure, the rate of blood flow through the kidney, or the dilution of the blood colloids as measured in the last four experiments by the proportion of corpuscles. It appears from these considerations that the amount of sulphate eliminated by the kidney is not determined by any single factor, and the different factors which we recorded vary independently of each other and that to such an extent that it is impossible to state whether their total activity is sufficient to explain the differences in the rate of elimination of sulphate. And the glomerular filtrate calculated from the sulphate excreted necessarily shows similar variations. Thus in the last four experiments the hæmatocrite readings may be taken as indicating the dilution of the colloids of the blood and gave a corpuscular content of 32.5, 30, 28.5 and 27. But the corresponding rates of renal blood-flow were 11, 35.3, 13.6 and 28.7 c.c. per minute respectively, and the filtrate is calculated at 2.22, 9.33, 1.45 and 3.59 c.c. The blood dilution and the rate of flow each must

exercise an important influence on the amount of filtrate, but how far their variations suffice to explain those in the filtrate it is at present impossible to state. The uncontrollable factor is the rate of flow.

The results in Exps. 4 and 7 are particularly discordant. In these experiments the collection of urine was postponed for an unusually long time after the operation had commenced. While there is still a moderate amount of filtration in progress, the relative loss of fluid from the blood is strikingly small. When, however, the records of carotid pressure and renal blood-flow are examined, it is evident that one would be justified in expecting a considerably more rapid passage of glomerular filtrate than is shown to have occurred; for the systemic pressure is not remarkably low, and the local circulation is good. We cannot, therefore, regard the comparative loss of efficiency in the work of the kidney as a result either of surgical shock or of renal vaso-motor disturbance, and are compelled to suppose that an increase has taken place in the resistance of the filtering membrane. This probably denotes an insufficient supply of oxygen to the glomerulus, and suggests that smaller changes in resistance may be physiologically produced by variations in oxygen supply. These alterations would be sufficient to modify the effects of the recognised physical factors which are concerned in filtration. We believe that alterations in the permeability of Bowman's membrane are of normal occurrence, and may be of considerable importance in controlling the rate of excretion by the kidney.

CONCLUSIONS.

(1) The glomerular filtrate, or the proportion of plasma which comes into efficient contact with the excretory cells, is from 20 to 25 p.c. of the whole plasma circulating through the kidney, but this proportion is subject to wide variations, sometimes rising to nearly 40 or falling to 6 p.c.

(2) These variations cannot be regarded as due to any one of the factors governing the activity of the kidney, and the interaction of these factors is so complicated that it is impossible to state that they are together sufficient to explain the variation. It is suggested that in addition to the known physical factors the permeability of the glomerular capsule may also vary under physiological conditions.

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EFFECTS ON THE CIRCULATION OF CHANGES IN THE CARBON-DIOXIDE CONTENT OF THE BLOOD.

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RECENT years have produced much discussion of the phenomena of acapnia and their possible relation to conditions of shock. Yandell Henderson⁽¹⁾, in a series of papers has dealt with the circulatory failure and general loss of vitality produced (by excessive artificial respiration continued for long periods in the dog. Moore⁽²⁾, Haldane⁽³⁾, and Henderson⁽⁴⁾ and his colleagues in their more recent writings, have attributed the phenomena to a supposedly pernicious effect of "alkalosis"—this term being used to mean an actual increase of alkalinity, a fall of the hydrogen-ion concentration of the blood. On the other hand, there have been some attempts to prove that the phenomena described by Henderson owed their origin to purely mechanical embarrassment of the circulation by the vigorous ventilation of the lungs. There is also a growing body of evidence, which has even had notable additions while our own observations have been awaiting record, which points to a special function of carbon dioxide as such, apart from the changes in hydrogen-ion concentration which accompany variations in its concentration, as a stimulus to the respiratory centre in particular. Our work started with the observation of the ease and rapidity with which the arterial pressure in the cat is depressed by excessive artificial ventilation of the lungs, and the rapidity with which recovery takes place when spontaneous respiration, or merely adequate artificial ventilation is resumed. A phenomenon with such immediate onset and rapid disappearance, capable of practically indefinite repetition, was obviously more suitable for experimental analysis than that studied by Henderson⁽¹⁾ in dogs, apparently needing periods up to several hours for its production. The phenomena are almost certainly related; possibly what we have studied is the initial reaction which leads ultimately to the condition studied by Henderson. We are not concerned here with the relation of the phenomenon to shock. We are dealing, as was Henderson apparently, with a response to ventilation of such excessive vigour as no

voluntary or reflex effort could achieve, and the meaning of the immediate effect of this on the circulation of the cat is our subject.

Methods. We have used cats almost entirely. A few experiments on rabbits showed the same phenomena in a rather less striking form. The animals were anaesthetized with full doses of urethane or paraldehyde, unless they had been decerebrated under chloroform or ether. In the earlier experiments we used an ordinary, single-action respiration pump (Brodie-Palmer). Later, and with results on the whole more satisfactory, we used a double-barrelled pump, with a system of sliding valves actuated by a revolving cam, devised for the purpose by our colleague Dr E. Schuster⁽⁵⁾ on the general lines of the suction and thrust pump of Hans Meyer, but made to work with the gear furnished with the Palmer pump. Hydrogen-ion concentrations of the blood were determined by the modified dialysis method which we recently described⁽⁶⁾, alkali reserves by Van Slyke's apparatus.

The effect on the systemic arterial pressure. When an anaesthetized cat, breathing naturally or under slow and gentle artificial respiration, is over-ventilated by the application of artificial respiration of adequate amplitude, and of a frequency from 100 to 180 strokes per minute, the carotid arterial pressure begins to fall in a few seconds, and reaches a low level, often 30–40 mm., in one or two minutes. The effect is the same whether air, oxygen, or an oxygen-nitrogen mixture with 7 p.c. oxygen is employed. Not infrequently the operation of mechanical factors is apparent at the moment of commencement of the accelerated ventilation; the precise effect of these factors no doubt varies according as the animal was previously breathing naturally or not, and according to the relation between the phases of the natural respiration, the artificial respiration and the heart beat. In the great majority of cases, however, the mechanical conditions operate to produce a sudden drop in the arterial pressure, shown on the tracing by a nearly vertical descent (Fig. 1). The pressure then continues to fall more slowly and usually attains a steady low level in 1 to 2 minutes; less frequently the commencement of artificial respiration is signalled by the appearance of a sharp upward peak, which is succeeded by a rapid descent of non-mechanical origin; very often, as will be apparent later, the mechanical effect is quite trifling. The low arterial pressure thus produced by over-ventilation persists, and may even fall further, while the excessive ventilation is maintained, but in some cases partial recovery occurs, or a slow, small, wave-like rhythm of rise and fall in the pressure appears. When the excessive ventilation is stopped, and a slow rate of artificial

respiration is substituted, or the resumption of natural respiration is allowed, the pressure is rapidly restored to the original level. With the animal breathing spontaneously, a period of one or two minutes' apnoea follows the cessation of the rapid ventilation, but fatal or even prolonged apnoea has not been observed, even after excessive ventilation for over half an hour.

Although the over ventilation cannot satisfactorily be carried out when volatile anæsthetics are given, there is no reason to suppose that the nature of the anæsthetic materially influences the results. Thus it is immaterial whether paraldehyde (Fig. 1) or urethane (Fig. 2) is employed. Nor is the manner of effecting the artificial ventilation a matter of great consequence, in our earlier experiments the respiration was performed with a single blast pump and tracheal tube with side opening, but in the later experiments the double action (suction and thrust)

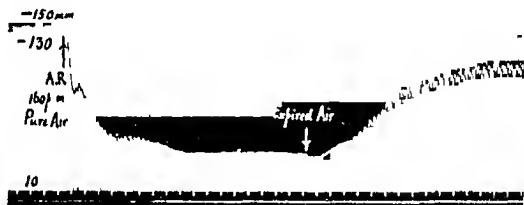


Fig. 1

pump was employed, with almost identical results. By this latter method of ventilation it is possible conveniently to operate with the opened thorax, and the fact that, under such conditions, the same effects on blood pressure are seen, serves to show that the phenomenon is not merely the result of mechanically produced changes in the intrathoracic pressure. The clearest demonstration that mechanical causes are inoperative, or only remotely operative, in producing the main fall of blood-pressure, is given by substituting ordinary human expired air, containing 4-5 p.c. CO_2 and drawn by the pump from a Douglas bag, for pure air, in carrying out the excessive ventilation. If such ventilation is begun with expired air the phenomenon remains in abeyance, or is confined to a trifling fall of pressure due to mechanical factors, but the characteristic depressor effect is at once evoked on substituting the ordinary air of the room for the bag of expired air, if, on the contrary, the blood pressure has first been lowered by rapid ventilation with pure air, it is speedily

restored to a normal level (or to a level which, owing to mechanical effects, may be a little lower than the normal) when expired air is substituted under identical conditions (Figs. 1, 2, etc.). The production of the depression by pure air and its reversal by expired air may be repeated indefinitely. The effect which we are considering is, therefore, primarily chemical in its origin, and due to excessively rapid removal of CO_2 from the system, as Henderson maintained concerning the effect which he described. There are two main questions before us. (1) Through action on what organ is the collapse of the arterial pressure produced? (2) What is the exact nature of the chemical change which produces it?

1. *Location of the effect.*

Effect on the heart. The changes in the heart's action accompanying excessive ventilation are so small that they can play little, if any, part in producing the fall of arterial pressure. Henderson observed an acceleration of the pulse-rate as the pressure fell in his experiments on dogs. We have seen acceleration in some, retardation in other experiments on the cat with intact vagi, the particular effect produced being often reversed when the vagi were divided. Thus, in one experiment, when the vagi were intact, the rate of the heart beat became slower, as the pressure fell under excessive respiration with pure air, and quickened again as the pressure was restored under ventilation with expired air. These effects were reversed when the vagi were cut, the pulse quickening with the fall and becoming slower with the rise. Obviously, the cardio-regulating centres are affected, and the effect is that of the withdrawal of CO_2 from the system, not a reflex from the mechanical effect on the lungs. The effect is not constantly present, however. In another experiment with intact vagi excessive ventilation with pure air caused quickening of the heart as the pressure fell. This is the normal reflex effect of a fall of arterial blood-pressure, when produced by some influence which does not affect the centres directly. In this same experiment, renewal of excessive ventilation with pure air, after the vagi had been divided, caused a slight fall in the rate of the heart-beat, which might have been due to removal of a slight tonic action of the accelerator centre, or to direct effect on the heart. It is evident that we have here a complex of opponent effects, the resultant action varying with the excitability and state of tonic activity of the centres. All the effects, however, are weak, and the net result on the heart rate is far too small to be a serious factor in the change of arterial pressure.

The same is true of the effect of excessive ventilation on the output

of the heart. On the heart-lung preparation (dog) Patterson (7) showed that administration of CO_2 caused reduction of the amplitude, increase of the diastolic volume, and slower rhythm, the result being reduction of the minute-volume. The change of aortic pressure, which occurs in the whole animal in our experiments, alters the conditions. We made a few experiments, therefore, with the cardiometer, and found that when, in a cat under rapid artificial respiration, pure air was substituted for expired air, the consequent fall of arterial pressure was associated with a small reduction in mean heart volume, a small reduction of output per beat, but an acceleration of rate which almost completely compensated for these changes; so that the resultant change in output per minute was a reduction so small that it could not be a serious factor in the fall of arterial pressure. The following are the figures obtained in such an experiment.

Exp. 1.

Rapid artificial respiration	Rate of beat	Ventricular output per beat	Output per minute
With pure air	164	0.6 c.c.	99 c.c.
„ expired air	158	0.65 „	103 „

It was of special importance to establish the fact that the fall of arterial pressure was not due to action on the heart, since Moore and Whitley (8), as the result of experiments on the isolated rabbit's heart, perfused with oxygenated Locke-Ringer solution, had drawn rather far-reaching conclusions as to the effect of excessive ventilation in producing "alkalosis," and thereby causing shock. Moore drew attention to the fact that in the ordinary Locke's solution it is customary to put only 0.01-0.02 p.c. of sodium bicarbonate, instead of the 0.25 p.c. which would represent the accepted proportion in blood-plasma. With a solution containing the latter proportion, and vigorously aerated with a current of air or oxygen, he found that the heart soon failed to beat. We made a number of experiments with artificial perfusion-fluids, in which the reaction could be adjusted by adding acid or alkali, or varying proportions of CO_2 . We used two solutions, both containing the usual proportion of KCl (0.042 p.c.), CaCl_2 (0.024 p.c.) and dextrose (0.1 p.c.). The first contained 0.84 p.c. NaCl and 0.06 p.c. Na_2HPO_4 . The reaction as prepared was pH 7.3-7.5, and it could be made more alkaline to the extent required by adding the appropriate amount of NaOH. The second solution contained 0.25 p.c. NaHCO_3 and the NaCl was correspondingly reduced to 0.7 p.c. The reaction could be adjusted by passing oxygen through the main bulk of the solution and CO_2 through a small portion. By adding varying small quantities of the latter to the main bulk, a

range of reactions between pH 7.2 and pH 8.5 could be obtained. The latter was the reaction obtained by prolonged oxygenation of the fluid, without addition of CO_2 . The fluid so treated deposited microscopic crystals of CaCO_3 on standing overnight. This was also about the limit of alkalinity for the solution containing the phosphate, which at this reaction began to become turbid with tricalcium phosphate.

On a series of rabbits' and cats' hearts our results were quite concordant. With fluids of alkalinity up to about pH 8 the hearts beat rather better on a more alkaline than a less alkaline fluid. This was especially the case with hearts which had been beating a long time. So that, up to a limit which, as we shall see, lies near to the maximum of alkalinity attainable in the blood of the living animal by simple over-ventilation, even the isolated heart, beating in Locke's solution, is not unfavourably affected. Beyond this range again, at about pH 8.3-8.5 another phenomenon appears. The heart is initially stimulated by the change to such a reaction, but it is soon evident that the outflow of the perfusion fluid, with constant inflow pressure, is becoming rapidly smaller. This change is soon followed by reduction in the amplitude of the beat, and after a few minutes the rate of perfusion has become very small and the heart slow and feeble. This reduction in perfusion-rate was noticed by Moore, and apparently regarded as secondary to the failure of the beat. We are convinced that the obstruction to perfusion caused by contraction of the coronary arterioles is the primary phenomenon. It appears before any reduction in the amplitude of the beat can be detected. The nature of the secondary depression of the activity of the intrinsic heart muscle is not far to seek. The most perfectly oxygenated Locke's solution carries no excess of oxygen to the beating mammalian heart. Any reduction in the rate of flow causes deficiency of oxygen supply, and Moore's phenomenon can be faithfully reproduced by simply reducing the perfusion pressure, without changing the reaction of the perfusion fluid from the normal optimum. The phenomenon is produced by the conditions of perfusion with saline solution: it does not occur within a range of alkalinity such as excessive ventilation can produce *in vivo*; and we shall see that, even when the alkalinity *in vivo* is raised to a point beyond the maximum attainable in the perfusion fluids, nothing like this phenomenon is produced. Moore's observation has, therefore, no relation to our problem.

Changes in volume and viscosity of the blood. In several conditions of low blood-pressure which have been recognized as having the characteristics of shock a pronounced decrease in the volume of circulating blood

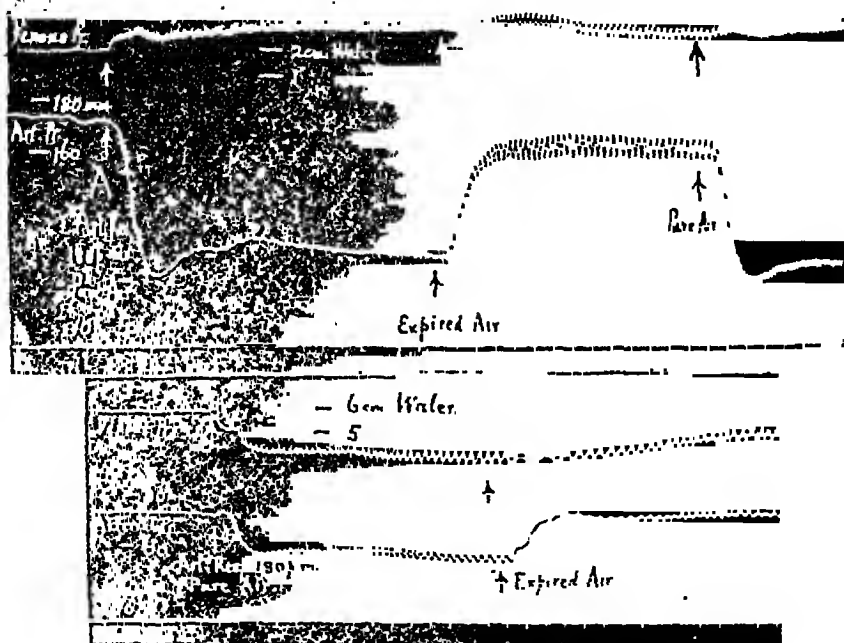
has been observed, largely due to the loss of plasma from the vessels. Henderson has described this condition as occurring in dogs after over ventilation for some hours. It did not seem credible that a change of this kind could occur with sufficient rapidity to account for the acute depression which we were studying, and actual observation showed that such change as accompanied it was in the opposite direction. The only change which was detected in the corpuscular content of the blood was a small decline, such as normally occurs as a secondary result of a fall of blood pressure(9). Such a compensatory dilution has the effect of simultaneously increasing the volume and lowering the viscosity of the blood. The shrinkage of the red corpuscles accompanying removal of CO_2 (Hamburger(10), Price Jones(11)) would also slightly lower the viscosity. These changes in volume and viscosity would tend to neutralize one another in their effects on the arterial pressure. In any case they are altogether too small to be regarded as material factors in the fall of pressure, of which they are clearly results and not causes. The following indicates the magnitude of the change.

Exp 2 Cat Urethane, double vagotomy			
Time min	B P mm	Hb p c in arterial blood	Remarks
0	120	86	Natural respiration, ventilation at 180 p m begun
1	86	86	
4½	68	78	
7½	70	76	
10	66	Expired air given at 160 p m	
11½	98		80
15	96		86
17	144	87	Artificial respiration off. Natural respiration begins 1 min later

The effect on venous and pulmonary pressures. If the minute volume of blood expelled by the heart is unchanged at the commencement of the over-ventilation, while the arterial pressure falls as a result of a lowering of the peripheral resistance, it would be expected that the venous pressure would fall slightly, and that this fall of diastolic filling pressure would secondarily reduce the minute output, though the reduction might be compensated by an increase of pulse rate. If the effects on the venous pressure are purely mechanical however, then, provided that a double action pump is applied, either a rise or a fall of venous pressure might result, without at the same time producing much alteration in cardiac output.

In experiments in which the venous pressure was measured, the effect was variable, as would be expected from the interference of the several

antagonistic influences, but the change of venous pressure, whatever its direction, was never great. The results of an experiment are shown in Figs. 2 and 3. Fig. 2 shows the venous pressure record from the lower end of the vena cava in an urethanized eviscerated cat, with both vagi divided. When double-action artificial respiration at the rate of 180 per minute was applied, the sharp fall in arterial pressure was accompanied by a rise of venous pressure equal to about 1 cm. of water. This effect was presumably chiefly mechanical. On changing over to expired air at the same rate of ventilation there was a very small and transitory rise



Figs. 2 and 3.

of pressure (about $\frac{1}{2}$ cm. water) following the rise of arterial pressure, and is another very small, brief rise on returning to pure air. These effects are, in any case, inadequate to explain the changes in arterial pressure. After the thorax of this animal had been opened (Fig. 3) the immediate prodromal effect of over-ventilation was to produce a sudden fall of arterial pressure; this was succeeded by a slower fall, probably secondary to the fall in arterial pressure. When expired air was given the venous pressure rose again to about 1 cm. of water. These changes in venous pressure are in accordance with the observations of Cannon and Harvey (12), in discussing the causation of the

shock-like effects in dogs, refer to a mechanism which Henderson has called the veno-pressor mechanism. This they consider to consist in a change in the calibre of the small venules, which respond to chemical alterations in the blood within them; acapnic blood they believe to cause a constriction of these venules with consequent lowering of the venous pressure and venous inflow into the heart; blood containing excess of carbon dioxide, on the other hand, is believed to relax the venules and so reverses the effects of acapnia. In the experiments on the decapitated cat, which they bring forward in evidence of this hypothesis, concentrations of CO_2 of 15 to 20 p.c. were used, and it might with reason be objected to these, that such concentrations effect a considerable weakening of the heart, as was shown by Patterson (*l.c.*), for the heart-lung preparation, and by Mathison (13) for the spinal cat. The relatively large

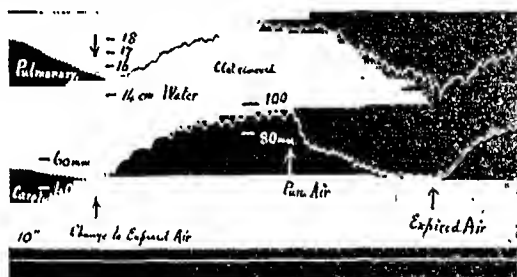


Fig. 4.

rise of venous pressure of 6 cm. of saline, which Henderson and Harvey saw, would thus seem to be explicable as due to depression of the heart. We do not think, therefore, that there is at present sufficient justification for invoking such a special mechanism as that proposed by Henderson to account for the phenomenon now under consideration, since the change of venous pressure and cardiac output witnessed under the conditions of our experiments, when physiological concentrations of carbon dioxide are administered, are too trivial to explain the large effects on the systemic arterial pressure.

The pulmonary arterial pressure may be regarded in a sense as a mirror of the pressure and velocity of blood in the great veins and the left auricle, and we have, therefore, made a few observations on pulmonary pressure by Schäfer's method (14). The results were in

produced. This is certainly not the case. In a series of 15 experiments, in which the alkalinity of the blood was raised by the intravenous infusion of sodium bicarbonate, we saw nothing in any way comparable with the change in the arterial pressure, which accompanies the production of a similar alkalinity by excessive ventilation. Two experiments may be quoted:

Exp. 8. Cat. 1.8 kg.; ether; natural respiration.

Time h. m.	c.c. of 6 % NaHCO ₃ intravenously	Arterial pressure mm.	Arterial blood.		Hbg %	Rate of respiration
			pH	Alk. res. (plasma) %		
0 0	—	148	7.25	48	76	40
	Infusion begins					
0 13	38 c.c. (in 13')	120	7.75	156	60	26
0 35	—	111	7.63	113	69	29

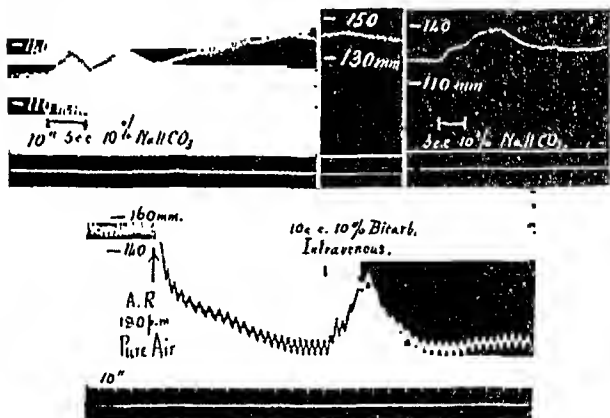
Exp. 9. Cat. 2.75 kg.; ether; natural respiration.

0 0	—	124	7.22	40	60	—
0 5	Infusion begins	—	—	—	—	—
0 35	30 c.c. (in 30')	108	—	—	—	—
0 37	—	110	7.73	80	54	—

It should be noted that, though respiratory rate and volume showed some reductions during such infusions, apnoea was never produced.

It might be suggested that the alkalinity so produced is neither so extreme, nor so suddenly developed, as that resulting from excessive ventilation. The objection is easily answered. While in experiments like the above, long infusion, with repeated bleeding for samples, does produce eventually a slight fall in the arterial pressure, no such fall is occasioned by the rapid injection of a large dose of bicarbonate, so as to cause a more sudden development of a higher alkalinity. Further, the sudden injection of such a dose of bicarbonate during the maintenance of excessive ventilation with pure air, does not produce a further fall of the arterial pressure, but only a temporary rise, in spite of the extreme alkalinity of the blood which is produced under such conditions. Fig. 10 shows the effect of injecting successive doses, each of 5 c.c. of 10 p.c. sodium bicarbonate, during the maintenance of gentle artificial respiration at a rate of 70 per minute. The immediate effect of the first and third injections is shown, and an intermediate section of the record, taken after the second injection. The total quantity of 15 c.c. having been injected, a sample of arterial blood was taken, and its reaction was pH 8.12. Fig. 11 shows the effect of injecting 10 c.c. of 10 p.c. bicarbonate in another cat, during the maintenance of excessive ventilation with pure air. Three minutes after this injection the artificial respiration was discontinued. The blood-pressure immediately began to rise, and, after

2 minutes' apnoea, natural respiration recommenced and was regular and normal half-a-minute later, when the blood pressure was 156 mm. The administration of 10 c.c. of 10 p.c. bicarbonate was repeated during a further period of over-ventilation, and again, in spite of the fact that the arterial blood must have been extremely alkaline, apnoea was of short duration ($1\frac{1}{2}$ minutes), and, when the blood-pressure was 108 mm., was succeeded first by shallow, and then, 3 minutes later, by normal respiration. A blood sample during this normal respiration, when the blood-pressure was 148 mm., showed an arterial pH of 7.80, which is much more alkaline than the normal. In order to form some idea of



Figs. 10 and 11.

what the reaction of the arterial blood must have been during the over-ventilation, this was reapplied, but without any further administration of bicarbonate. In 3 minutes an arterial sample showed a pH of 8.23. It is surprising, and quite at variance with the most widely-accepted views on the nature of the stimulus to the respiratory centre, that the cessation of artificial respiration, with the blood at this extreme alkalinity, should be followed by such a brief period of apnoea. The periods of apnoea, in fact, appeared to get rather shorter as the alkalinity of the blood increased. Table III gives the record of a similar experiment, and shows the extreme alkalinity which may be produced by combining

excessive ventilation with injection of alkali, with effects on the blood-pressure which are certainly not greater than those produced in the animal with initially normal blood-reaction.

Exp. 10. Cat. 2.1 kg.; urethane.

Time min.	B.P.	pH arterial blood	Respiration	Remarks
0	122	7.62	Natural	10 c.c. 10 % NaHCO ₃ rapidly, intravenous
1	140	—	"	—
2	100	7.98	"	—
14	90	—	"	10 c.c. more
16	110	8.00	"	—
17	—	—	Start A.R. at 180 per min.	—
41	48	9.00	After 24 min. A.R.	A.R. stopped
43	82	7.88	Apnoea	20 sec. before natural respiration begun
	(rising)			
45	146	—	Natural	—

Just as an increase of bicarbonate reserve of the blood, although associated with a more alkaline reaction of the plasma, in no way essentially modifies either the arterial pressure, or the responses of this to a period of excessive ventilation, so a reduction of the alkali reserve, and a more acid blood than normal, leaves the response of the arterial pressure to ventilation unchanged, as the following record shows.

Exp. 11. Cat. 3.25 kg.; urethane; vagi cut. Arterial pressure 154 mm.; pH of arterial blood = 7.31. Injection during 21 mins. of 25 c.c. $n/2$ HCl into the femoral vein; the arterial pressure first rose, then fell to 114 mm. The reaction of the blood from this time onwards was too near the neutral point to allow accurate measurement to be made with the indicator used (neutral red). Spontaneous respiration continued but there was some dyspnoea. Rapid artificial respiration was commenced. In 1½ mins. the blood pressure fell from 114 to 34 mm.; the reaction was still near the neutral point. Expired air was substituted for atmospheric air, and the arterial pressure rose steadily and in 5 mins. was 112 mm.

The experiment showed that at a range of reaction which, at every stage, was well on the acid side of the normal reaction of blood, the depressor effect of removing CO₂ and the restorative effect of its re-introduction appeared in a perfectly normal manner.

These results make it clear that mere changes in the pH of the blood-plasma do not produce the phenomenon which we are discussing. Variations of reaction within wide limits may be produced, with surprisingly little effect on the state of the circulation, provided the changes are effected by adding fixed alkali or fixed acid to the blood. It is only when CO₂ is swept out of the system that the characteristic depression of vasomotor tone occurs; and, though this necessarily entails an increase of alkalinity, it is equally effective, whether the reaction is initially so acid that the loss of CO₂ fails to raise the alkalinity to the normal level,

or initially so alkaline that the alkalinity under rapid ventilation reaches a level which has usually been regarded as incompatible with life

These results are in line with a number of observations which have appeared during recent years, and which seem to indicate that free carbon dioxide as such, and apart from the increase of H ions produced by its addition to the blood, has a specific stimulant action on the respiratory centre (Scott(30), Collip(31), Hooker, Wilson and Connett(32)) The same would appear to be true of the bulbo spinal centres which are responsible for the maintenance of normal tone in the peripheral blood vessels Removal of CO_2 from the circulating blood depresses them, and, at the same time, normally lowers the H-ion concentration, replacement of CO_2 restores their activity, while the H ion concentration rises again But the action is due, in common parlance, to changes in 'the concentration of free CO_2 ,' not to the changes in the H ion concentration of the blood

The meaning of this specific effect of CO_2 , as distinguished from acidity, is by no means clear Many writers have considered the function of carbonic acid in the blood in terms of that very small fraction which is electrolytically dissociated into H and HCO_3 ions If this is the correct attitude, and if the undissociated CO_2 is to be regarded as physiologically indifferent, the effects which we are considering can only be explained if we suppose that a simultaneous rise of H and HCO_3 ions alone acts as a stimulus to the centres, their simultaneous diminution being necessary for producing the depressor phenomenon which is our subject Injection of alkali, which increases the HCO_3 ions while depressing the H ions, is accordingly ineffective, injection of fixed acid, which raises the H ions without increasing the HCO_3 ions is likewise ineffective What seems to be a more helpful suggestion, however, has been made by Jacobs(33), who, on the basis of experiments on the effects of CO_2 and fixed acids on tadpoles and infusoria, and on the sensation of sour taste, concludes that undissociated carbonic acid has a peculiar efficacy on account of its special property of penetrating cells On Jacobs' view, living cells are not readily permeable by H or HCO_3 ions, but the undissociated acid enters with relative ease, and, becoming partially dissociated in the interior, there raises the H ion concentration far more effectively than it can be raised by an external medium containing excess of H ions, but not of CO_2 Others have adopted a slightly different conception, according to which the presence of free CO_2 "sensitises" the nerve cells to H ions, presumably by affecting their permeability For such a process there is a rough analogy in the well known Zunz-

Hamburger effect of CO_2 on blood; the presence of free CO_2 in the plasma causes H and Cl ions to enter the corpuscles. On such a theory of the effect of CO_2 on nerve cells, one might expect its efficacy as a stimulus to be conditioned by the pre-existent H-ion concentrations in the blood; but the results of our own experiments on the vasomotor centres, and those of Scott (*l.c.*), on the respiratory centre, suggest that the effect is largely independent of the reaction existing before the CO_2 is applied. It is possible, however, that the nerve-cells readily adjust themselves to a new reaction-equilibrium with the fluids surrounding them, and that it is the disturbance of this which constitutes the effective stimulus. Our experiments do not throw any new light on this question of the intimate mechanism of the action. Their result is merely to add, to the recent evidence concerning the rhythmic activity of the respiratory centre, evidence that the tonic action of the vasomotor centres is likewise conditioned by the concentration of free carbon dioxide rather than of hydrogen ions in the arterial blood. Briefly, the fall of arterial pressure, which has been the subject of this investigation, is the result of "acapnia," in the original sense given to the term by Mosso, as accepted by Henderson in his earlier papers dealing with excessive ventilation, and not of "alkalosis" as more recent speculations have suggested.

CONCLUSIONS.

The phenomenon studied is the fall of arterial pressure in the cat when excessive, rapid artificial respiration is applied, and its recovery when the same rate of respiration is maintained with expired air. It has been found:

(1) that these effects are due to depression and recovery of the vasomotor centres of the bulb and spinal cord;

(2) that they are due to abstraction and replacement of free carbon dioxide, and not to changes in the hydrogen-ion concentration of the blood.

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ACID PRODUCTION IN SHED BLOOD.

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THE carbon dioxide capacity of blood is at its highest level in freshly drawn blood, and usually suffers considerable reduction when the blood is allowed to stand out of the body. Although this observation was made by Zuntz as long ago as 1868(1), and though recent work has enlarged our knowledge of the conditions under which the phenomenon occurs, no satisfactory explanation of it has yet been proposed. Separated plasma does not undergo this change, and the speed of the change in whole blood is accelerated by temperature elevation. All observers are agreed that the phenomenon is irregular in incidence and magnitude. Human venous blood usually shows it well, though occasionally there is no change even after several hours at room temperature. The results obtained by Christiansen, Douglas and Haldane(2) showed that the change may occur with considerable but progressively diminishing velocity while blood is being brought into equilibrium with gas mixtures at body temperature. Extrapolation and interpolation of their values indicates that during successive periods of 20 minutes the CO_2 capacity fell about 2.5 p.c., 1.8 p.c., 1.1 p.c., 0.8 p.c. and 0.7 p.c. This slowing-down of the speed of change I have also been able to confirm. As I have previously pointed out(3), this post-mortem diminution of the available base of the blood offers a serious obstacle to the interpretation of data obtained from blood which is not perfectly fresh. Peters, Barr and Rule(4) have also encountered, but partially circumvented, this same difficulty in their determinations of arterial CO_2 tensions from the CO_2 dissociation curve and the arterial CO_2 content of the blood. Henderson and Haggard(5), however, and Mellanby and Thomas(6) have shown independently of one another that the velocity and extent of the irreversible change are greatly exaggerated in blood which has been temporarily exposed to a lowered carbon dioxide pressure, and Mellanby and Thomas(6) further showed that the change so produced was associated with, and presumably caused by, an increase in the lactic acid content of the blood.

It is evident from the data that the cause of this autogenous acid production is to be sought in the formed elements of the blood, and is not due to bacterial action. In searching for its cause it occurred to me that it might well be associated with the phenomenon of glycolysis. This is likewise of irregular occurrence, is associated with the formed elements (chiefly the leucocytes, according to Maclean and Weir(7)), is accelerated by alkali (Cl Bernard(8)) and, therefore, by removal of CO_2 (Rona and Doblin(9)). Moreover, the rate at which glycolysis occurs is most rapid in fresh blood and is subsequently retarded (Edelmann(10)). If we remember that glycolysis is associated with a conversion of glucose into lactic acid, as shown by Slosser(11), Kraske, Kondo and v. Noorden(12), such an explanation seems most probable. I have, therefore examined and, as I think, confirmed this hypothesis by experiment.

Methods Samples of human blood were drawn from suitable arm veins into serum syringes provided with appropriate anticoagulants. Dog's and goat's blood was drawn in a similar way from the external jugular vein. The blood of cats (decerebrated) was drawn from an arterial cannula and that of rabbits from the ear vein. As anticoagulants, oxalate 0.1 to 0.5 p.c., or hirudin, were employed, in some cases the blood was defibrinated. In order to retard the change in blood the following procedure was adopted in the earlier experiments. Two thermos flasks were filled, one with ice and salt and one with ice and water. In the first of these a boiling tube filled to a depth of 1-2 cm. with liquid paraffin was cooled down to about -12°C , the fresh drawn blood was run straight into this, beneath the surface of the paraffin, and stirred with a thermometer until its temperature had fallen to 0° when the tube with its contents was at once transferred to the second flask where it was kept at 0° till required. About five minutes is required thus to cool 10 or 20 c.c. of blood from body temperature to 0° . If loss of a little CO_2 is immaterial, the paraffin layer may be omitted, the boiling tube being then closed by a rubber bung. An advantage of this method of cooling is that the blood can, if necessary, be transported in the cold condition and the thermos flask kept on the laboratory bench while determinations are made. Keeping at 0° , however, does not always entirely prevent change if many hours elapse. A further difficulty is that, as soon as the cooled blood is warmed up again, as in the usual procedure for saturation with a known pressure of CO_2 , the change again sets in with rapidity. In the earlier experiments this was minimised by carrying out the equilibration at a low temperature, $20-22^\circ$, in later ones by

addition of sodium fluoride, as will presently be described; the latter procedure enables equilibration with gas mixtures to be carried out safely at body temperature. For this purpose, a mixture of one part of sodium fluoride and four parts of potassium oxalate, both in fine powder, was used and 0.3 to 0.5 p.c. of the mixture added to the blood, which was at once cooled as described above.

Carbon dioxide contents were determined by Van Slyke's method. In some cases dissociation curves were plotted. More usually, economy in time and material was effected by the determination of one point only, viz., the CO_2 content at approximately alveolar CO_2 tension, the actual CO_2 pressure being in each case accurately determined by analysis of the gas after equilibration. A part of the CO_2 dissociation curve was then plotted for one sample of the blood, and the proper small corrections made from this for the reduction of all the various determinations to the same pressure of CO_2 . Blood sugar determinations were made by Benedict's method. Although the question of vital importance concerns the extent of the autogenous acid production in blood kept for a *short* time at room temperature, or equilibrated at body temperatures for periods up to 20 minutes, I have in these experiments not infrequently warmed blood to 37° for periods of one to three hours or left it at room temperature for longer intervals, in order to get more extensive changes. Bacterial action, may of course, play some part in these, but has not been separately studied.

The extent of the change. Effect of temperature. As stated above, the appearance of the phenomenon is irregular, and its extent depends especially on temperature, CO_2 loss, etc. Rarely, in spite of apparently favourable conditions, the change is insignificant. There may even be a slight increase in CO_2 capacity; as this was seen most strikingly in blood to which urea had previously been added, I imagine it to be due to a measurable production of ammonia, the content of which is known to rise in blood on standing. But in the great majority of cases when blood is allowed to stand, even at room temperature, there is, in an hour or two, a loss of CO_2 capacity of one or two volumes per cent.; less frequently, the reduction may amount to 6 vols. p.c. or even more. Exp. 1 is an illustration of the magnitude of the change under favourable conditions, when the blood is partially depleted of CO_2 while still in the body.

Exp. 1. Decerebrate cat submitted to excessive artificial respiration. Arterial blood drawn and 0.2 p.c. oxalate added to it. A portion of this cooled at once to 0° was found from its dissociation curve to have a CO_2 content of 37.3 vols. p.c. at 40 mm. CO_2 and

37.5° C. A portion of the blood allowed to stand for 1½ hours at 20° C and then similarly cooled to 0° till required, had under the same conditions a CO₂ content of 33.8 vols p.c.—a loss of 3.5 vols p.c. The content of CO₂ of the arterial blood, as drawn, was 32.5 p.c. From the dissociation curve for the fresh blood the CO₂ pressure in the arterial blood, as drawn, would, therefore, be about 27.5 mm, that deduced from the stale blood however, would be about 36.5 mm—a difference of 9 mm.

Fig. 1 shows an experiment with human blood.

Relation between fixed and free CO₂ If the change in shed blood is due, as Mollanby and Thomas's results would lead us to suppose, to a formation of fixed acid, then it should be possible to show that, if the blood, previously brought to a low CO₂ pressure, is kept out of contact

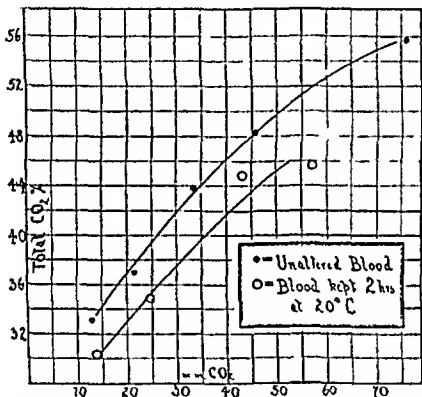


Fig. 1. CO₂ dissociation curves of fresh (fluoride) human blood, and of the same without fluoride after two hours' exposure to air at 20° C

with the air, the free CO₂ rises in proportion as the fixed CO₂ falls. Experiments were made as follows. The CO₂ content of the blood was lowered, either *in vivo* in cats by the carrying out of excessive artificial respiration, or for human blood *in vitro* by rapid evacuation at a low temperature (18–20° C). The blood was either oxalated (.15 p.c.) or hirudinised. A 20 c.c. all-glass syringe, containing a glass bead for mixing, was completely filled with the blood, sealed, and kept at the desired temperature. Samples were withdrawn at intervals for determinations of the CO₂ pressure by Krogh's micro method (13). In Exp. 2 there was, even after 2½ hours, no reduction in the fixed CO₂ content of the blood, and in agreement with this the only change in CO₂ tension

given amount of fixed acid in the blood, would be considerably smaller than that calculated on the assumption that the acid was entirely used in replacing fixed CO_2 . As a matter of fact, I have already shown⁽³⁾ that when the CO_2 capacity of blood falls during its stay *in vitro*, this change chiefly affects the corpuscles by altering what Haggard and Henderson⁽⁵⁾ call their "acid load," while leaving the true plasma but little changed.

Before a comparison between the change of sugar content and CO_2 capacity can be satisfactorily made, it is, therefore, necessary to know the effect on the CO_2 capacity, of the addition to blood of varying amounts of fixed acid. I have accordingly tested this point by adding to fluoride

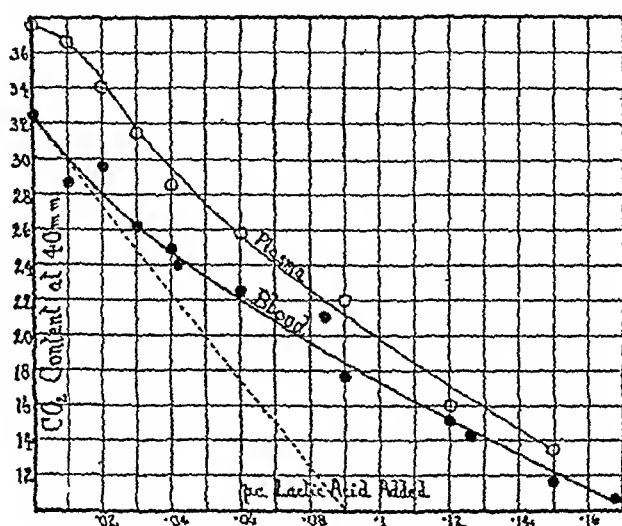


Fig. 4. Effect of lactic acid on the CO_2 content of blood and of true plasma, at 40 mm. CO_2 and 38°C .

blood amounts of lactic acid varying, in stages, from 0.01 to 0.17 p.c. The blood so treated was in each case equilibrated at 38°C . with CO_2 at 40 mm. and the CO_2 content of whole blood and of true plasma determined. The results, which are given in Fig. 4, clearly bear out the anticipations referred to above. The straight dotted line indicates how the blood should behave if the added acid only replaced fixed CO_2 . The curves obtained show that the curve for whole blood departs a good deal, and that of true plasma even more, from the behaviour required by such a supposition. This is a further instance of the important secondary buffering, which the blood owes to its red corpuscles. If allowance is made for the fact that in the experiment which these curves represent, all the samples of blood were diluted 20 p.c. (with saline

solution and lactic acid in varying proportions), it is clear that the effect of this secondary buffering in undiluted blood, depending as it does on the corpuscular content (Evans(3)), would be still greater than that found here. This experiment bears out the results of Mellanby and Thomas(6), who also found that the effect of addition of lactic acid was smaller than might have been expected.

We may now consider the results of the experiments given graphically in Fig. 3 in which glycolysis and fixed acid production in blood were determined simultaneously. The straight dotted line is the hypothetical curve which would be expected if fixed CO_2 alone were displaced, while the curved dotted line gives the one that would be expected if the effect of the acid were that found from Fig. 4. It will be seen that the points fall a little below this curve, but appear to lie on a similar curve parallel to it. As these experiments were made with bloods from different sources, and all of them undiluted, I think the inference is clear. It is that the fall of CO_2 capacity is due to the almost quantitative conversion of each molecule of glucose lost by glycolysis, into two molecules of some monobasic acid, presumably lactic acid (or, less probably, one molecule of a dibasic acid).

The effect of mere lowering of free carbon dioxide on the change. It might be inferred from the experiments of Haggard and Henderson(5) and of Mellanby and Thomas(6) that it is the mere reduction of the free CO_2 which in some way at once leads to the irreversible fall of CO_2 capacity of the blood. According to my view, however, all that the removal of CO_2 does is to produce a hydrogen-ion concentration so low as greatly to accelerate the normal process of glycolysis. Exp. 5 shows that this is so.

Exp. 5. Fresh rabbit's blood (0.2 p.c. of potassium oxalate) was cooled down to 0° . As a control 0.05 p.c. of NaF was added to a portion of it. The remainder was evacuated at the low temperature of 18°C. , as thoroughly as possible with a filter pump, and then divided into four equal parts: to No. 1 0.05 p.c. of NaF was added at once; No. 2 was warmed to 38°C. for 5 min.; No. 3 for 30 min.; and No. 4 for 3 hours; and then 0.05 p.c. of NaF was added to each. The CO_2 content at 40 mm. and the sugar content of each sample was then determined, with the results given in the table.

Treatment of blood					CO_2 content	Sugar content
					p.c.	p.c.
Fresh	49.0	0.247
Evacuated at 18°C.	49.0	0.250
"	"	warmed	5 min.		46.5	0.234
"	"	"	30 min.		45.0	0.232
"	"	"	3 hrs.		34.0	0.129

This experiment shows that the change is not produced by mere removal of CO_2 , but that it sets in very rapidly on warming *after this* has been done.

given amount of fixed acid in the blood, would be considerably smaller than that calculated on the assumption that the acid was entirely used in replacing fixed CO_2 . As a matter of fact, I have already shown(3) that when the CO_2 capacity of blood falls during its stay *in vitro*, this change chiefly affects the corpuscles by altering what Haggard and Henderson(5) call their "acid load," while leaving the true plasma but little changed.

Before a comparison between the change of sugar content and CO_2 capacity can be satisfactorily made, it is, therefore, necessary to know the effect on the CO_2 capacity, of the addition to blood of varying amounts of fixed acid. I have accordingly tested this point by adding to fluoride

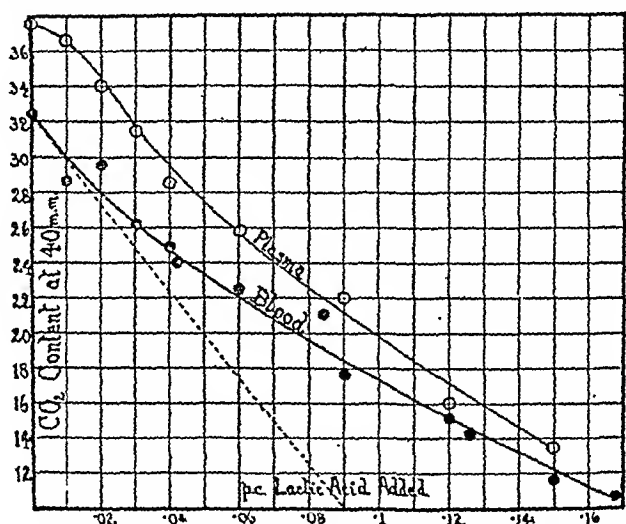


Fig. 4. Effect of lactic acid on the CO_2 content of blood and of true plasma, at 40 mm. CO_2 and 38°C .

blood amounts of lactic acid varying, in stages, from 0.01 to 0.17 p.c. The blood so treated was in each case equilibrated at 38°C . with CO_2 at 40 mm. and the CO_2 content of whole blood and of true plasma determined. The results, which are given in Fig. 4, clearly bear out the anticipations referred to above. The straight dotted line indicates how the blood should behave if the added acid only replaced fixed CO_2 . The curves obtained show that the curve for whole blood departs a good deal, and that of true plasma even more, from the behaviour required by such a supposition. This is a further instance of the important secondary buffering, which the blood owes to its red corpuscles. If allowance is made for the fact that in the experiment which these curves represent, all the samples of blood were diluted 20 p.c. (with saline

solution and lactic acid in varying proportions), it is clear that the effect of this secondary buffering in undiluted blood, depending as it does on the corpuscular content (Evans⁽³⁾), would be still greater than that found here. This experiment bears out the results of Mellanby and Thomas⁽⁶⁾, who also found that the effect of addition of lactic acid was smaller than might have been expected.

We may now consider the results of the experiments given graphically in Fig. 3 in which glycolysis and fixed acid production in blood were determined simultaneously. The straight dotted line is the hypothetical curve which would be expected if fixed CO_2 alone were displaced, while the curved dotted line gives the one that would be expected if the effect of the acid were that found from Fig. 4. It will be seen that the points fall a little below this curve, but appear to lie on a similar curve parallel to it. As these experiments were made with bloods from different sources, and all of them undiluted, I think the inference is clear. It is that the fall of CO_2 capacity is due to the almost quantitative conversion of each molecule of glucose lost by glycolysis, into two molecules of some monobasic acid, presumably lactic acid (or, less probably, one molecule of a dibasic acid).

The effect of mere lowering of free carbon dioxide on the change. It might be inferred from the experiments of Haggard and Henderson⁽⁵⁾ and of Mellanby and Thomas⁽⁶⁾ that it is the mere reduction of the free CO_2 which in some way at once leads to the irreversible fall of CO_2 capacity of the blood. According to my view, however, all that the removal of CO_2 does is to produce a hydrogen-ion concentration so low as greatly to accelerate the normal process of glycolysis. Exp. 5 shows that this is so.

Exp. 5. Fresh rabbit's blood (0.2 p.c. of potassium oxalate) was cooled down to 0° . As a control 0.05 p.c. of NaF was added to a portion of it. The remainder was evacuated at the low temperature of 18°C ., as thoroughly as possible with a filter pump, and then divided into four equal parts: to No. 1 0.05 p.c. of NaF was added at once; No. 2 was warmed to 38°C . for 5 min.; No. 3 for 30 min.; and No. 4 for 3 hours; and then 0.05 p.c. of NaF was added to each. The CO_2 content at 40 mm. and the sugar content of each sample was then determined, with the results given in the table.

Treatment of blood				CO_2 content	Sugar content
				p.c.	p.c.
Fresh	49.0	0.217
Evacuated at 18°C	49.0	0.250
"	"	warmed	5 min.	40.5	0.234
"	"	"	30 min.	45.0	0.234
"	"	"	3 hrs.	34.0	0.234

This experiment shows that the change is not produced by CO_2 , but that it sets in very rapidly on warming after

that there is, under these conditions, both a leucocytosis and a hyperglycæmia. I have found that the addition of glucose to blood decidedly accelerates this change. Under such conditions, delay in the cooling of blood samples, and lack of proper precautions to prevent the change, may result in grave errors. I am not prepared to suggest to what extent these changes affect the oxygen-dissociation curve of blood, but that there is some effect seems inevitable.

SUMMARY.

1. The fall in CO_2 capacity of shed blood is due to a conversion of glucose into lactic acid as a result of glycolysis.
2. The change is greatly accelerated, though not actually produced, by a lowering of the CO_2 pressure of the blood, as shown by Mellanby and Thomas, and Henderson and Haggard.
3. These facts are of importance in all experiments on the CO_2 dissociation curve. The change can be retarded by adding 0.05–0.1 p.c. of sodium fluoride to the blood. Equilibrations at body temperature can then be performed with little change in the CO_2 capacity of the blood.

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ON THE HYDROGEN-ION CONCENTRATION AND SOME RELATED PROPERTIES OF NORMAL HUMAN BLOOD¹.

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THE object of this paper is to establish, on a number of normal persons, the relations existing between the hydrogen-ion concentration (eH) and certain other properties of human blood.

1. *The eH— $p\text{CO}_2$ relation.* Many authors have published curves relating the eH of the plasma (or serum) to the CO_2 -pressure ($p\text{CO}_2$) to which the blood is exposed. The published curves differ considerably from one another, but it is uncertain to what extent these differences are due to systematic errors in the various methods used, and to what extent they are due to individual differences in the bloods of the subjects. A study of the bloods of ten normal persons, by the same methods and the same observers, should show how far such variations are natural, and should establish a good "average" relation for normal man.

2. *The $p\text{CO}_2$ — $v\text{CO}_2$ relation.* This has been studied by a variety of authors, Jaquet(1), Bohr(2), Christiansen, Douglas and Haldane(3), but there is no "average" curve available, and no systematic statement² of the extent to which the CO_2 -dissociation curve may vary in a series of individuals.

3. *The eH— $v\text{CO}_2$ relation.* The steepness of the curve relating the volume of CO_2 absorbed ($v\text{CO}_2$) to the H-ion concentration measures the degree to which the blood is buffered. This also has been determined on a number of normal persons, and an "average" has been fixed.

4. *The K —eH relation.* The value of K in the equation,

$$y = Kx^n/(1 + Kx^n),$$

¹ These experiments were planned by Barcroft, as part of a larger scheme, and carried out by him together with Bock, Parsons, Parsons and Shoji. The ms account was left with Hill when Barcroft sailed on the Scientific Expedition to Peru, in Nov. 1921. It was prepared for publication by Hill, who has added a certain amount of theoretical matter.

² (Added in proof.) See however van Slyke(18).

of the dissociation curve of blood, is known to change with the H-ion concentration of the plasma. The connection between K and plasma- cH , in a variety of normal individuals, and in the "average" normal man, is of interest in itself, and throws light upon the equilibria existing, and the interchanges occurring, between plasma and corpuscles.

5. *The K — pCO_2 relation.* The relation between K and the CO_2 pressure is necessarily deducible from (4) and (1) above. Actually it is the one which has been directly studied experimentally.

6. *The K — rCO_2 relation.* The relation existing between K and the volume of CO_2 absorbed is necessarily deducible either from (4) and (3), or from (5) and (2) above.

Subjects.

Name	Nationality	Sex	Occupation
Barcroft	British	M	Physiologist
Bock	U.S.A.	M	Research student
Collis	British	M	Undergraduate (athletic)
McLean	"	M	Undergraduate
Parsons, T. R.	"	M	Lecturer
Parsons, W.	"	F	Married woman
Porter	"	F	"
Priestman	"	M	Undergraduate
Redfield	U.S.A.	M	Research student
Shoji	Japanese	M	" "

1. *The cH — pCO_2 relation.*

The H-ion concentration has been measured on reduced blood by means of the hydrogen-electrode, as described by Parsons(4). In the following the H-ion concentration is expressed in terms of $10^8 \times cH$: by this means, without rendering the results unintelligible by the use of a logarithmic notation, the whole range is kept within the limits of the numbers 1-10.

TABLE I.

Barcroft		Bock		Collis		Parsons, T. R.		Parsons, W.	
pCO_2	$10^8 \times cH$	pCO_2	$10^8 \times cH$	pCO_2	$10^8 \times cH$	pCO_2	$10^8 \times cH$	pCO_2	$10^8 \times cH$
20.6	2.95	19.8	1.51 ?	20.7	2.45	17.6	2.45	20.4	2.57
30.3	3.55	29.8	3.09	33.1	3.16	31.5	3.16	30.6	3.31
38.6	3.71	40.8	3.71	40.3	3.71	41.3	3.47	40.2	4.17
49.1	4.47	41.3	3.71	49.8	4.27	—	—	—	—
		50.6	4.47	58.1	4.79	71.5	4.07	59.6	5.01
Priestman		Shoji		Porter		Redfield		McLean	
pCO_2	$10^8 \times cH$	pCO_2	$10^8 \times cH$	pCO_2	$10^8 \times cH$	pCO_2	$10^8 \times cH$	pCO_2	$10^8 \times cH$
20.5	2.29	19.9	2.63	20.1	2.45	19.2	2.45	19.3	2.34
31.0	2.88	30.3	3.47	20.5	2.45	29.8	3.09	28.9	3.09
39.8	3.47	40.8	4.17	32.0	3.31	39.2	4.07	38.1	3.47
49.2	3.98	48.0	4.47	44.3	3.98	39.6	3.80	57.3	4.57
56.0	4.27	59.3	5.25	46.0	4.17	48.8	4.36		
				59.5	4.85				

The results for each individual if plotted in a diagram such as Fig. 1, give a curve which is almost indistinguishable from a straight line. By careful analysis of all the results together it can be shown that the relation is *not* exactly linear, but slightly concave to the $p\text{CO}_2$ axis, as in Fig. 1. The individual curves vary slightly in position and slope, but apart from the observations on T. R. Parsons (which are anomalous) all the observed lines lie within the comparatively narrow limits shown. The curve of Fig. 1 represents the "average" relation, for all the subjects, calculated as follows: all the observations in the neighbourhood of 20 mm.

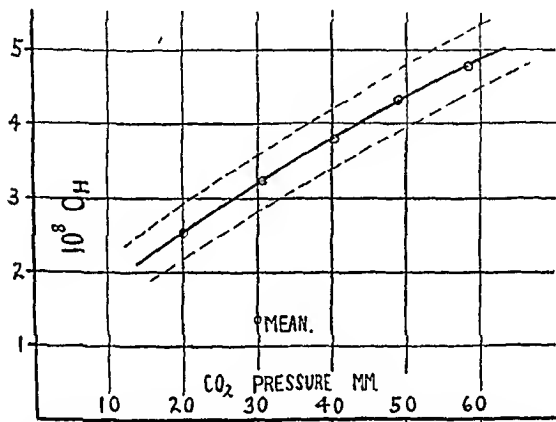


Fig. 1 Relation between $c\text{H}$ and $p\text{CO}_2$. The heavy line represents the relation for the "average" normal man, the broken lines enclose the relations of nine normal persons.

CO_2 -pressure are taken, and their average $c\text{H}$ plotted against their average $p\text{CO}_2$: the same is repeated for the observations in the neighbourhoods of 30, 40, 49 and 58 mm. CO_2 -pressure, with the following result:

Mean CO_2 pressure	20.1	30.6	40.3	48.8	58.3
Mean $c\text{H} \times 10^8$	2.52	3.22	3.80	4.31	4.79

So far therefore as readings with the hydrogen electrode represent true values of the H-ion concentration these mean numbers, represented by the curve of Fig. 1, give us a good average of the $c\text{H}$ — $p\text{CO}_2$ relation in normal man. The relation is nearly linear, being slightly concave however to the $p\text{CO}_2$ axis.

cH-values. Table II therefore gives the relation between H-ion concentration and volume of absorbed CO_2 , for eight normal subjects.

TABLE II.

Barcroft		Bock		Collis		McLean		Porter	
rCO_2	10^8 cH	rCO_2	10^8 cH	rCO_2	10^8 cH	rCO_2	10^8 cH	rCO_2	10^8 cH
38.0	2.95	41.4	1.51?	38.2	2.45	38.0	2.34	38.2	2.45
43.5	3.55	47.6	3.09	46.2	3.16	42.0	3.09	38.4	2.45
46.8	3.71	52.1	3.71	49.0	3.71	45.5	3.47	44.5	3.31
50.5	4.47	52.9	3.71	52.5	4.27	51.7	4.57	49.5	3.98
		56.8	4.47	56.2	4.79			50.0	4.47
								54.5	4.85
Priestman		Redfield		Shoji					
rCO_2	10^8 cH	rCO_2	10^8 cH	rCO_2	10^8 cH				
44.0	2.88	37.0	2.45	37.5	2.63				
48.5	3.47	45.0	3.09	43.0	3.47				
52.0	3.98	50.0	4.07	48.0	4.17				
54.5	4.27	50.2	3.80	57.0	5.25				
		54.1	4.36						

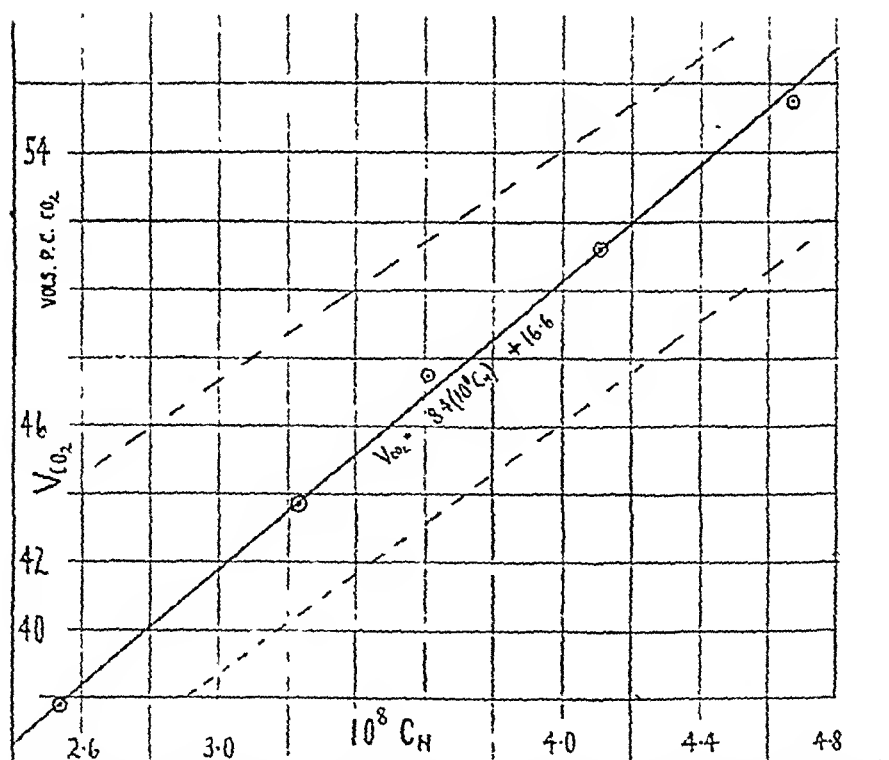


Fig. 3. Relation between rCO_2 and cH. The heavy line, which is exactly straight, denotes the relation for the "average" normal man, the broken lines enclose the relations for eight normal persons.

The results for each individual, if plotted in a diagram such as Fig. 3, give a curve indistinguishable from a straight line. Careful analysis in this case shows that over the range considered the relation is as nearly linear as could be desired. The individual lines vary somewhat in position and slope, but all lie between the broken lines of Fig. 3. The heavy curve of Fig. 3 represents the mean relation for all the subjects calculated as follows: all the observations in the neighbourhood of 38 volumes per cent of CO_2 are taken, and their mean tCO_2 plotted against their mean cH ; the same is repeated for the observations in the neighbourhood of 44, 48, 51 and 55 volumes, with the following result:

Mean vol per cent CO_2	37.8	43.7	47.1	51.2	55.1
Mean $\text{cH} \times 10^8$	2.51	3.23	3.61	4.11	4.67

These five points are shown by circles in Fig. 3, and it is seen that they lie on a straight line. It is obvious of course that the relation cannot be linear throughout its whole length, but it is a fortunate chance which makes it so exactly linear within the limits of physiological importance: within these limits any variation from a straight line is negligible, and the average line for normal man may be taken as having the equation

$$\text{tCO}_2 = 8.4 (10^8 \text{cH}) + 16.6 \quad (\text{I})$$

This equation, together with the other deduced above viz.,

$$10^8 \text{cH} = 1.7 p\text{CO}_2 / \text{tCO}_2 \quad (\text{II}),$$

enables us to calculate any of the relations already discussed by simple algebra. For example, eliminating tCO_2 between equations (I) and (II), we find,

$$p\text{CO}_2 = 1.79 (10^8 \text{cH})^2 + 3.57 (10^8 \text{cH}),$$

from which we may calculate the following:

10^8cH	2.5	3.0	3.5	4.0	4.5
$p\text{CO}_2$ calcd	20.1	26.8	34.4	42.9	52.3
$p\text{CO}_2$ obsd (Fig. 1)	20.0	27.0	34.0	43.0	52.5

No better agreement could be desired. Or again, eliminating cH between equations (I) and (II) we find,

$$(\text{tCO}_2)^2 - 16.6 (\text{tCO}_2) = 39.5 p\text{CO}_2$$

from which we may calculate the following:

tCO_2	40	45	50	55
$p\text{CO}_2$ calcd	23.7	32.3	42.3	53.3
$p\text{CO}_2$ obsd (Fig. 2)	21.7	30.1	41.5	54.0

Here again the agreement is good. These two simple equations, with different constants, are probably of quite general character over the range of physiological importance.

Equation (I) we may write in the more general form,

$$v\text{CO}_2 = b (10^8 c\text{H}) + c \dots\dots\dots(\text{III}),$$

where b and c are constants. Of these constants, b is of fundamental interest and importance, since it is a measure of the completeness with which the blood is buffered: for a given increase in $c\text{H}$, the increase in $v\text{CO}_2$, *i.e.* the extra CO_2 absorbed, is proportional to b . The constant c is not of the same importance; it determines simply the absolute quantity of CO_2 taken up by the blood.

From the data of Table II equations have been calculated similar to equation (III), and the constants of this equation are as given below in the eight individuals concerned.

Name	b	c	Name	b	c
Barcroft	10.1	9	Porter	6.9	21
Bock	6.7	27	Priestman	7.8	21
Collis	7.9	19	Redfield	9.5	13
McLean	6.5	23	Shoji	8.1	15

Thus the "average" normal man has a b of 8.4, and in normal persons b varies between 10.1 and 6.5, which we may describe roughly by writing $b = 8.4 \pm 2$: while the "average" normal man has a c of 16.6, and in normal persons c varies from 9 to 27, which we may describe roughly by writing $c = 16.6 \mp 10$: it will be noticed that a large c usually accompanies a small b , and *vice versa*.

In this quantity b we have a simple expression of that most important physiological factor, the degree to which the blood is buffered. It will be interesting to find how far the normal limits of b , 8.4 ± 2 , are exceeded in abnormal or sick persons, or in persons accustomed to severe exercise or to low oxygen pressures. At present we will not concern ourselves more with this side of the subject, except to point out that only by using the rational expression $c\text{H}$, and discarding the artificial logarithmic notation, is it possible to find relations of the simplicity and intelligibility of those discussed above. For a further discussion see Appendix II.

4. The effects of acid and CO_2 on the oxygen-dissociation curve.

It has long been known that the O_2 -dissociation curve of blood is affected by CO_2 and acids, and for some years that this effect is fairly

sharply localised on the constant K in Hill's equation $y = Kx^n/(1 + Kx^n)$, of the dissociation curve. In 1911 Barcroft and Peters(7) showed that there is a linear relation between pH ($-\log cH$) and $\log K$, and Hasselbalch(6), who called this relation the "P—B" line, showed that the "P—B" line was straight for the blood of certain other individuals, and at first supposed that it was invariable in position. In the case of abnormal persons, however, he found that the line, while still straight, is not always in the same place. The same was found by Donegan and Parsons(9).

Again, it was pointed out by L. J. Henderson(10) that if $1/K$ he plotted against the CO_2 -pressure, for certain published curves of Barcroft's blood, the result is a straight line. Adair(11) independently made the same discovery, and further noted that what is true of the effect of CO_2 on the oxygen-dissociation curves of blood is equally true of its effect on the CO dissociation curves.

These facts have recently been discussed from the theoretical standpoint by A. V. Hill(12),(13), his theory is confirmed by the fact that, without further hypothesis, it allows us to calculate quantitatively the diminution in the CO_2 absorption caused by the oxygenation of blood(3). We will return to the theory later.

We have made a number of experiments on the relation between K and the CO_2 -pressure, as follows. About 2 c.c. of blood is put into a saturator vessel of about 250 c.c. capacity, with a mixture of oxygen and nitrogen calculated to give about half saturation. When equilibrium has been established at $37^\circ C$, two or sometimes three samples of blood (0.10 to 0.15 c.c.) are withdrawn into the smaller form of differential apparatus, and the percentage saturation determined. The oxygen and CO_2 -pressures in the saturator are also observed, by the abstraction of a sample of gas for analysis. Sufficient CO_2 is then introduced to give the next required tension of that gas, and sufficient oxygen again to give about half-saturation. When the equilibrium is again attained samples for analysis are taken as before, and the whole process is repeated. By the time two points have been determined there will probably be little blood left in the saturator, and it will be necessary to introduce more. The number of observations which should be made without the introduction of fresh blood is a matter of some importance. If sufficient blood be put into the saturator at the start to serve for the determination of six points, it is probable that before the end of the experiment the blood will have become more acid. The last portion will have been incubated six times, or 70 minutes in all. In some cases we used the same blood

for three consecutive points, but in the later experiments only for two.

The following example will give an idea of the pressures of gas used, and the accuracy of the blood-gas determinations.

Pressure of CO_2	3	10	19	41	67	99
Pressure of O_2	17	18	22	28	31	35
Percentage saturation with O_2	73	60	52	54	52.5	50
	70	62	51	52	49	48
	71	—	—	—	—	52

The results have been expressed in terms of the relation between $1/K$ and $p\text{CO}_2$, and data for the ten subjects considered are given in Table III. In the calculation of $1/K$ it has been assumed throughout that $n = 2.5$, a point which will be considered later.

TABLE III.

Barcroft		Bock		Collis		McLean		Parsons, T. R.	
$p\text{CO}_2$	$1/K$	$p\text{CO}_2$	$1/K$	$p\text{CO}_2$	$1/K$	$p\text{CO}_2$	$1/K$	$p\text{CO}_2$	$1/K$
3	515, 430, 500	2.0	660, 715	2.5	880, 860, 730	1.5	550, 595	4.0	870, 810, 650
10	910, 830	11.4	1350	11.2	1350, 1410	11.5	1670, 1800	9.6	1050, 1360
19	2010, 2190	17.8	2100, 2450	16.6	2000, 2670	26.7	3200, 3480	17.0	1810, 1660, 1950
41	3360, 3720	39.2	3140	38.0	4000, 3660	40.4	4220, 4400	35.5	2700, 2900
67	4550, 5280	57.5	4740, 4250	57.0	5100, 5000	62.3	5700, 5900	58.0	4450
99	6880, 7230, 7350	89.0	8220	88.0	7980, 8330	95.0	9550, 9880	86.5	5750, 6170
Parsons, W.		Porter		Priestman		Redfield		Shoji	
$p\text{CO}_2$	$1/K$	$p\text{CO}_2$	$1/K$	$p\text{CO}_2$	$1/K$	$p\text{CO}_2$	$1/K$	$p\text{CO}_2$	$1/K$
2.6	370, 460, 380	1.0	490	3.5	420, 490, 450	1.9	495, 495	3	660, 650
12.2	1980, 1590	12.7	1530, 1550	8.7	600, 940	9.3	1350, 1350	10	1340
19.5	2560, 2300	25.3	2700, 2940	20.5	1280, 1700, 1960	23.3	2270	22	2370, 2370
36.6	3020, 2660	43.5	3960, 3720	39.8	3690	44.2	3900, 4090	40	3250, 3020
61.7	5480, 5630, 5310	68.6	7770, 7060	56.0	5150, 6660, 8070	50.0	4870, 5090	60.5	5650, 5150
90.6	8350, 8470							100	8170, 8730, 8850

The results for each individual, if plotted in a diagram such as Fig. 4, give a curve which (as Adair and Henderson found) is very similar to a straight line. On examining the curves closely, however, the evidence will be found to be cumulative that the relation is really an S-shaped curve of the type shown in Fig. 4. The individual curves vary slightly in position and slope, and are not shown in the figure. The left-hand curve of Fig. 4 represents the average relation calculated as follows (the right-hand curve we shall refer to later): all the observations in the neighbourhood of a CO_2 -pressure of 2.5 mm. are taken and their mean $1/K$ plotted against their mean $p\text{CO}_2$; the same is repeated for the observations in

the neighbourhood of 11, 20, 40, 60 and 90 mm. CO_2 -pressure, with the following result:

Mean $p\text{CO}_2$	2.5	10.7	20.1	39.8	60.4	92.5
Mean $1/K$	586	1330	2340	3510	5090	8010

In taking the mean in the neighbourhood of 60 mm. the observations on Porter were not included, as being abnormally large and probably in error, Redfield was left out as not having a point sufficiently close to 60 mm., and Priestman's value was taken as 5150 which is more

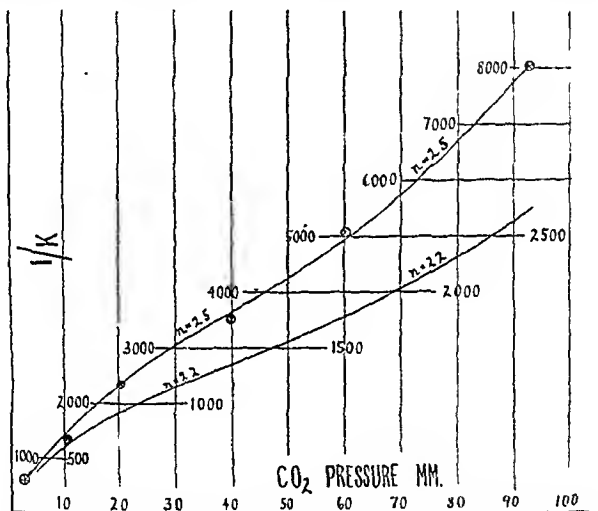


Fig. 4. Relation between $1/K$ and $p\text{CO}_2$. The left hand curve represents the relation for the "average" normal man, with a $1/K$ calculated for $n \approx 2.5$. The right hand curve represents the same, calculated for $n = 2.2$. The vertical scales of the two curves are different and as marked. Note that the relation is approximately, but not accurately, linear.

in keeping with the rest of the observations on him than his other two abnormally high readings.

These six mean points are shown with circles in Fig. 4, and a suitable S-shaped curve is interpolated between them. This curve denoted " $n = 2.5$ " may be useful as defining the dissociation curve of the "average" normal man at 37°C ., and at all CO_2 -pressures, assuming $n = 2.5$.

From the curves of Figs. 1 and 4 it is possible to calculate the relation between K and cH . We will proceed first to test the truth of the statement that $\log K$ is a linear function of pH . In Table IV are given values of cH and $1/K$ read off at a series of values of pCO_2 from Figs. 1 and 4 respectively. In rows (a), (b) and (c) are given the values of pCO_2 , $10^3 cH$ and $1/K$. In rows (d) and (e), $\log (10^3 cH)$ and $\log (1/K)$. In order to show that the two last named quantities are connected by a linear relation, we give in row (f) the quantity $0.88 \log (1/K)$ and in the last row the difference $0.88 \log (1/K) - \log (10^3 cH)$. It is seen that the numbers in the last row are very nearly constant, having a mean value of 2.559. Hence there is a linear relation,

$$0.88 \log (1/K) = \log (10^3 cH) + 2.559 \dots\dots\dots(IV),$$

between $\log (1/K)$ and $\log (10^3 cH)$, which confirms entirely, and very exactly, the previous conclusions of Barcroft and Peters(7), and of Hasselbalch(6).

TABLE IV.

(a) pCO_2	20	25	30	35	40	45	50	55	60
(b) $10^3 cH$ Fig. 1	2.50	2.87	3.19	3.51	3.82	4.11	4.38	4.63	4.87
(c) $1/K$ Fig. 4	2320	2710	3040	3360	3650	3940	4260	4610	4950
(d) $\log (10^3 cH)$.398	.458	.504	.545	.582	.614	.642	.666	.688
(e) $\log (1/K)$	3.366	3.434	3.483	3.527	3.562	3.596	3.630	3.664	3.695
(f) $0.88 \log (1/K)$	2.962	3.023	3.064	3.103	3.134	3.164	3.195	3.224	3.251
(f) - (d)	2.564	2.565	2.560	2.558	2.552	2.550	2.553	2.558	2.563

According to A. V. Hill's theory(12),(13) of the effect of acid on the oxygen-dissociation curve the relation between cH and $1/K$ should be capable of being expressed in the form

$$1/K = cH/kcB$$

where k is a constant, and cH and cB are the concentrations of hydrogen and basic ions inside the corpuscle. There is reason to believe that the H -ion concentration of the inside of the corpuscle is higher than that in the plasma, but proportional to it (see below). If so $1/K$ should be proportional to the cH of the plasma, and the relation written above should be

$$\log (1/K) = \log (10^3 cH) + \text{const.}$$

without the 0.88 multiplying the first logarithm. At first sight the very exact relation (IV) appears to negative, or to require some modification in, Hill's theory. It was pointed out, however, by Hill(13) that such an effect may result from the use of an incorrect value of n in calculating K . All the values of K have been calculated, with the assumption that $n = 2.5$, from observations at about half-saturation of the blood. Suppose

the true values of K and n to be K_0 and n , while the assumed values are K and 2.5. Then from the equation of the dissociation curve we have

$$y/(1-y) = K_0 x^n = Kx^{2.5}.$$

But the observations have been made at such oxygen and CO_2 -pressures as to give approximately half saturation, so that on the whole

$$y = \frac{1}{2} = 1 - y.$$

Hence $1 = K_0 x^n = Kx^{2.5}$. Taking logarithms we find:

$$(1/n) \log (1/K_0) = \log x = (1/2.5) \log (1/K).$$

Hence the true value $\log (1/K_0)$ is equal to $(n/2.5) \log (1/K)$, where n is the true value of n , and $1/K$ is the value calculated on the false assumption that $n = 2.5$. There is an accumulation of evidence that the value $n = 2.5$ is rather too great. Barcroft(8) summarised the observations on which the use of the equation $y/(1-y) = Kx^n$ is based, and from these observations the most suitable value of n has been recalculated, by means of a special diagram in which $\log [y/(1-y)]$ is plotted against $\log x$. If the equation is obeyed the relation is linear, and the slope of the line (which gives n) may be very accurately read off. The following results are obtained:

Douglas (38 observations)	$n = 2.3$
Barcroft (21 observations)	$n = 2.4$
Zuntz (9 observations, 40 mm. CO_2)	$n = 2.5$
Zuntz (15 observations, 34-35 mm. CO_2)	$n = 2.15$
Haldane (16 observations)	$n = 2.6$

Of this series the observations on the blood of Douglas are far the most complete and satisfactory, and give a value of n definitely less than 2.5. Recent experiments (hitherto unpublished) in the Physiological Laboratory, Manchester, tend to confirm this. The shape of the dissociation curve (pig, sheep, ox) tends to give an n of about 2.2 (Brown); the ratio

$$\frac{(\text{beat of combination per grm. mol. of haemoglobin})}{(\text{beat of combination per grm. mol. of } \text{O}_2)}$$

tends to give an n of about 2.0 (Brown); the direct measurement of the osmotic pressure tends to give about 2.2 (Atkinson); while the ratio

$$\frac{(\text{excess of } \text{CO}_2 \text{ taken up by reduced blood at given cH})}{(\text{O}_2\text{-capacity of blood})}$$

gives 2.1 or 2.2 (Hill(13)). Let us suppose therefore that the true value of n is 2.2. Then

$$\log (1/K_0) = (2.2/2.5) \log (1/K) = 0.88 \log (1/K)$$

so that, replacing the quantity $0.88 \log (1/K)$ in equation (IV) by $\log (1/K_0)$, we find:

$$\log (1/K_0) = \log (10^3 cH) + 2.559 \dots\dots\dots(V).$$

Thus, assuming a value 2.2 for n the relation between $1/K$ and cH is exactly of the type predicted by Hill's theory. The exactness of the relation, and of the agreement with theory, is strong evidence for the general validity of the reasoning and methods adopted. In view of this it seems advisable in future to assume the value $n = 2.2$, or possibly 2.3, instead of $n = 2.5$, in calculations of the dissociation curve of normal human blood.

The right-hand curve of Fig. 4 gives the relation between $1/K_0$ and the CO_2 -pressure, K_0 being calculated for $n = 2.2$. This curve may be useful as defining the dissociation curve of the "average" normal human blood at 37°C ., at all CO_2 -pressures.

As regards variation of the K - cH relation from one individual to another, we give next Table V, showing the relation between K and cH for nine subjects. The numbers given are, in the first column $\log (10^3 cH)$, in the second column $\log (1/K_0)$ and in the third column

$$\log (1/K_0) - \log (10^3 cH).$$

In this K_0 is calculated for $n = 2.2$. The values of cH are those measured with the H-electrode and recorded in Table I: for a given cH the $p\text{CO}_2$ is shown in Table I, and the value of $1/K$ corresponding to this $p\text{CO}_2$ is read off from the subject's curve constructed from the data of Table III. Then $\log (1/K_0)$ is calculated¹ as $0.88 \log (1/K)$.

TABLE V. $a = \log (10^3 cH)$; $b = \log (1/K_0)$.

Barcroft			Bock			Collis			McLean			Parsons, W.		
a	b	$b-a$	a	b	$b-a$	a	b	$b-a$	a	b	$b-a$	a	b	$b-a$
4.47	2.96	2.49	4.3	3.04	2.61	3.9	2.93	2.54	3.7	3.01	2.64	4.1	2.96	2.55
5.5	3.06	2.51	5.5	3.12	2.57	4.9	3.09	2.60	4.9	3.13	2.64	5.2	3.08	2.56
5.7	3.09	2.52	5.7	3.21	2.64	5.7	3.17	2.60	5.4	3.20	2.66	6.2	3.09	2.47
6.5	3.16	2.51	6.3	3.19	2.54	6.3	3.22	2.59	6.6	3.29	2.63			
						6.5	3.30	2.65						

						Redfield			Shoji		
a	b	$b-a$	a	b	$b-a$	a	b	$b-a$	a	b	$b-a$
2.60	3.36	2.91	2.55	3.9	2.96	2.57	4.2	2.96	2.54		
3.08	2.56	4.6	3.05	2.59	4.9	3.06	2.57	5.4	3.04	2.50	
3.11	2.49	5.4	3.23	2.69	6.3	3.16	2.53	6.2	3.11	2.49	
3.32	2.64	6.0	3.20	2.60	5.8	3.15	2.57	7.2	3.20	2.48	
		6.3	3.26	2.63	6.4	3.21	2.57				

1. The relation is valid only on the assumption that K was calculated from observations in the neighbourhood of half-saturation. With other observations K_0 must be calculated from observations.

From Table V it is obvious that what is true of the "average" man is true also of the individuals making up the average viz that

$$\log (1/K_0) - \log (10^8 cH)$$

is constant, or in other words that $1/K_0$ is directly proportional to cH . The quantity $\log (1/K_0) - \log (10^8 cH)$ has the following mean values in nine subjects investigated.

Barcroft	2.51	Porter	2.57
Bock	2.59	Priestman	2.61
Collis	2.60	Redfield	2.56
McLean	2.64	Shoji	2.50
Parsons, W.	2.53		

The value given for the "average" man was 2.559, which is very nearly the same as the average of the values of the nine individuals.

The matter can be expressed most clearly without the logarithmic notation: the value of $1/K_0$, calculated for $n = 2.2$ from the equation of the oxygen-dissociation curve, is proportional to cH , the H ion concentration of the plasma, according to the equation,

$$1/K_0 = \alpha (10^8 cH) \dots\dots \quad (VI),$$

where in the "average" normal man the constant α has the value 360. In different individuals the constant α is different, having the following values for nine normal subjects:

Barcroft	324	Porter	372
Bock	389	Priestman	407
Collis	396	Redfield	363
McLean	436	Shoji	316
Parsons, W.	338		

We may summarise therefore as follows the information gained

(1) In human blood at 37° C. the best value to assume for n is rather less than 2.5: the value adopted here is 2.2.

(2) Calculated with this value of n , the value of $1/K$ is directly proportional to the H-ion concentration.

(3) In the "average" normal man the constant of this proportion is 360×10^8 : in normal persons it lies between 316 and 436×10^8 .

(4) The relation between $1/K$ and pCO_2 is approximately, but not exactly linear, when $1/K$ is calculated for $n = 2.5$ (Fig. 4, left-hand curve): for $n = 2.2$ the relation between $1/K_0$ and pCO_2 is definitely not linear (Fig. 4, right-hand curve).

We come finally to the very important problem of what it is which determines the value of K in human blood, and therewith the dissociation curve. K undoubtedly changes with CO_2 -pressure, and with cH , but at a given pCO_2 , or at a given cH , the value of K varies from

one individual to another. There is clearly some other factor at work. It is of course possible that the hæmoglobin of one individual is not the same in its properties as that of another. This subject, however, has already been investigated by Adair, Barcroft and Bock (14), who came to the conclusion, in spite of some superficial evidence to the contrary, that hæmoglobin must be reckoned as having identical properties in normal individuals of the same species. Neglecting this possibility, therefore, we come to the essential fact that the cH measured by any of our methods is that of the plasma, while K is the constant of a reaction occurring *inside the red blood corpuscle*. It seems possible that, even if the cH 's of the plasma be the same in two individuals, the cH 's of their corpuscular contents, and consequently their K 's, may be different. The experiments of Milroy (15), and certain hitherto unpublished experiments made by R. E. Conway at Manchester, tend to prove that the cH inside the blood corpuscle is indeed higher than that in the plasma, but there still remains the fundamental question of how such inequalities of cH can be produced. The fact that $1/K_0$ is proportional to plasma- cH is a proof that a change in plasma- cH produces a proportional change in the cH of the corpuscular contents, presumably by simple passage of the H -ions into the corpuscle. But if H -ions can pass freely through the corpuscular wall why should they not reach an equal concentration on the two sides? We believe the solution of this set of problems to lie in a Donnan "Membrane Equilibrium" (16), (17) occurring at the boundary of the corpuscle. In order to make the position clear it will be necessary to adopt different symbols to denote concentrations in the plasma and in the corpuscles respectively: we will use $C(H)$, $C(B)$, etc., to denote concentrations in the plasma, and $c(H)$, $c(B)$ etc., to denote concentrations in the corpuscles. Imagine that the corpuscle, in addition to its hæmoglobin, contains the same chemical bodies as the plasma but in different proportions, viz. Cl , HCO_3 , H , OH , phosphates P and basic ions B . Of these common constituents we will suppose that the *phosphates P and the basic ions B cannot diffuse through the corpuscular wall*, while the rest can. According to Donnan's thermodynamical reasoning, if there be *any* positive ion X capable of diffusing through the membrane, and *any* negative ion Y also capable of so diffusing, then we may write the equation,

$$C(X) \times C(Y) = c(X) \times c(Y)$$

for all such pairs of positive and negative ions. In our particular case therefore we may write

$$C(H) \times C(Cl) = c(H) \times c(Cl)$$

$$C(H) \times C(HCO_3) = c(H) \times c(HCO_3)$$

$$C(H) \times C(OH) = c(H) \times c(OH).$$

From these

$$\frac{C(H)}{c(H)} = \frac{c(Cl)}{C(Cl)} = \frac{c(HCO_3)}{C(HCO_3)} = \frac{c(OH)}{C(OH)} = (\text{addendo}) \frac{c(Cl) + c(HCO_3) + c(OH)}{C(Cl) + C(HCO_3) + C(OH)}.$$

Now of the total ionic charge inside the corpuscle hæmoglobin can provide, owing to its enormous molecular weight, only a negligible part. Compared therefore with the concentrations of basic, chlorine, phosphate and bicarbonate ions, the concentrations of hydrogen and hæmoglobin ions are negligibly small, so that the negative ions must have their charge balanced practically entirely by the positive basic ions, and we may write, for both sides of the membrane,

$$\begin{aligned} C(B) &= C(Cl) + C(HCO_3) + C(OH) + C(P) \\ c(B) &= c(Cl) + c(HCO_3) + c(OH) + c(P). \end{aligned}$$

Hence the above equation becomes

$$\frac{C(H)}{c(H)} = \frac{c(B) - c(P)}{C(B) - C(P)} \dots\dots\dots (VII).$$

Thus, any inequality in the difference between the concentrations of basic and phosphate ions on the two sides of the corpuscular envelope must necessarily lead to a proportional, but inverse inequality in the distribution of H-ions. In what follows we will assume for simplicity that the phosphate ions are at a concentration small compared with that of the basic ions: taking account however of the phosphate ions complicates, but does not change, the argument.

Now the fact that there exists a definite isotonic solution of NaCl, that a hypotonic solution lyses and a hypertonic solution shrinks the corpuscles, shows that NaCl (almost completely ionised as Na and Cl) cannot pass into the corpuscle. But the well known Hamburger effect, that Cl-ions can pass in and out, shows that the impermeability is not due to Cl-ions: it must be due to Na-ions. Hence we may expect to find inequalities of concentration of basic ions on the two sides of the corpuscular wall, and these inequalities will necessarily lead to inequalities of concentration of H-ions also, even though the latter can pass freely in and out. Such differences in the concentration of basic ions inside the corpuscle may well be the cause of all the observed variations in the constant α of equation (VI).

According to Hill's theory the value of $1/K_0$ is determined by the ratio $c(H)/c(B)$: but from equation (VII) $c(H) = C(H) \times C(B)/c(B)$, so that $c(H)/c(B) = C(H) C(B)/[c(B)]^2$. Thus according to the theory the value of $1/K_0$ should be directly proportional to the H-ion concentration of the plasma, as found experimentally to be the case, directly proportional to the basic ion concentration of the plasma (which

is practically constant and so cannot be the cause of any variations from one individual to another) and inversely proportional to the square of the basic ion concentration inside the corpuscle. Thus, comparatively trifling differences in the basic ion concentration of the inside of the corpuscle should be sufficient to provide all the differences found between different individuals: for example, in Shoji the constant α of equation (VI) was 316, and in McLean, 436, and this is the widest difference observed. The square root of the ratio 436/316, viz. 1.17, should be equal therefore to the ratio of Shoji's c (B) to McLean's c (B): that is to say, a 17 p.c. difference between the concentrations of basic ions inside the corpuscles of these two individuals would be sufficient to explain the observed difference in the effect of acid on their dissociation curves. We believe therefore that small natural differences, and possibly small progressive changes produced by time or by acclimatisation, in the basic ion concentration inside the red blood corpuscle, together with a Donnan membrane equilibrium at its boundary, are the cause of the differences in normal individuals of the way in which the H-ion concentration of the plasma affects the dissociation curve of blood.

SUMMARY.

The following relations have been established experimentally at 37° C. for the blood of the "average" normal man, and also the variations therefrom among ten normal persons:

1. The relation between plasma- cH (as measured by the H-electrode) and pCO_2 , the CO_2 -pressure: this is a slightly curved line (Fig. 1).

2. The relation between CO_2 -pressure and vCO_2 , volume p.c. of total absorbed CO_2 (Fig. 2). In the "average" normal man the empirical relation $cH = 4.7 pCO_2/vCO_2$ is accurately obeyed.

3. The relation between the volume p.c. of absorbed CO_2 and the H-ion concentration: see Fig. 3. This relation is exactly linear over the range of physiological importance, having the equation

$$vCO_2 = b \times (10^8 cH) \div c.$$

In the "average" normal man $b = 8.4$ and $c = 16.6$, while in normal persons the range of b is ± 2 , and of c ∓ 10 . The quantity b is of great importance, being a convenient measure of the degree to which the blood is buffered. This equation, together with that given in (2), enables us to calculate any one of the three relations required.

4. The relation between $1/K$ and CO_2 -pressure, K being the constant of Hill's equation, calculated for $n = 2.5$. This relation is not exactly

linear, as stated by Henderson and Adair, but slightly S-shaped (Fig. 4).

5. The relation between $1/K$ and eH . The existence of a linear relation between $\log 1/K$ and $\log eH$ is fully confirmed. $1/K$ is proportional therefore to some power of eH . According to Hill's theory $1/K$ should be proportional to the first power of eH : it is shown that this is accurately true if we assume a value of 2.2 for n instead of 2.5, a number which on other grounds also is preferable. If K_0 be the value of K calculated for $n = 2.2$, the equation:

$$1/K = \alpha (10^9 eH)$$

holds, both for the "average" normal man with $\alpha = 360$, and for nine individuals with α varying from 316 to 436.

6. The cause of these variations of α is discussed, that is to say the reason why, at a given plasma- eH , the dissociation curves of two individuals may be different. It is concluded that this is due to a Donnan membrane equilibrium occurring at the corpuscular envelope, differences of basic ion—phosphate ion concentration on the two sides of this membrane resulting in inverse differences of H-ion concentration, with consequent differences in the oxygen-dissociation curves.

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APPENDIX I.

The effect on the form of the dissociation curve, of the change in H-ion concentration produced by the oxygenation of blood. BY A. V. HILL.

The oxygenation of blood is known to cause a diminution in the CO_2 absorbed at a given CO_2 -pressure (3), and a rise in the $c\text{H}$ (4): conversely a rise in the $c\text{H}$ is known to diminish the degree of saturation with O_2 ; hence an increased degree of saturation tends, in virtue of the rise of $c\text{H}$ which it provokes, to hinder its own occurrence. If the change of $c\text{H}$ on oxygenation were sufficiently large this effect might considerably distort the shape of the dissociation curve; it is desirable therefore to examine how great the effect may be.

The change of $c\text{H}$ caused by oxygenating blood can be calculated approximately from the curve given by Christiansen, Douglas and Haldane (3, p. 256) employing the formula $c\text{H} = 4.7 p\text{CO}_2 / v\text{CO}_2$ given above. The oxygenation of the blood of J. S. H. may be shown to change its $c\text{H}$ by the following relative amounts at different CO_2 -pressures.

CO_2 pressure mm.	100	80	60	40	30	20	10
% increase in $c\text{H}$	10	11	11	12	12	13	14

The p.c. increase is nearly constant at all CO_2 -pressures. Parsons (4, p. 450) found in his own blood a constant decrease in $p\text{H}$, of 0.038 at all CO_2 -pressures, i.e. a constant 9 p.c. increase in $c\text{H}$: this is in fairly good agreement with the above. We will assume a 12 p.c. increase in $c\text{H}$ as the result of the change from complete reduction to complete saturation, and a proportional increase for partial saturation.

Consider the dissociation curve of blood maintained in some artificial manner (as by adding NaOH) at a constant $c\text{H}$, for all degrees of saturation. We shall assume the curve to obey the usual equation,

$$\frac{y}{1-y} = Kx^n \dots\dots\dots (1),$$

with an n of 2.2: we will then calculate the distortion of the curve, in the case of blood in which the $c\text{H}$ is allowed to increase naturally, as the degree of saturation is increased. In this case n will remain the same, viz. 2.2, while $1/K$ will alter somewhat from point to point of the curve. It has been shown in the preceding paper that $1/K$ is proportional to $c\text{H}$, so that $1/K$ will increase in the same proportion as $c\text{H}$. But the effect of a degree of saturation y is to increase $c\text{H}$ by $12y$ p.c.; hence it also

increases $1/K$ by $12y$ p.e., i.e. to $1/K(1 + .12y)$: or in other words K becomes $K/(1 + .12y)$. Hence equation (I) becomes:

$$\frac{y}{1-y} = \frac{Kx^n}{(1+.12y)} \text{ or } \frac{y(1+.12y)}{1-y} = Kx^n \dots\dots\dots\text{(II)}.$$

We will compare equations (I) and (II) numerically, assuming for simplicity that $K = 0.001$, a value corresponding to a CO_2 -pressure of about 25 mm., with $n = 2.2$. The result is as follows: similar effects occur for any other value of K .

y	.1	.2	.4	.6	.8	.9
x_I	8.5	12.3	19.2	27.6	43.3	62.6
x_{II}	8.6	12.4	19.6	28.7	45.2	65.7

We see, therefore, that a change is, in fact, produced by this effect: the curve is slightly stretched in the direction of the x -axis. The effect is not large, but it is not quite negligible. It can be shown that it results in an apparent small diminution in n , and an apparent small increase in K . If we attempt to fit the series of numbers, y and x_{II} , to a simple equation of the type (I), we find the most suitable equation to have an n of 2.16 (instead of 2.2) and a K of .001075 (instead of .001). The values of x calculated from equation (I) with these values of n and K , for the values of y given in the above Table, are, in order, 8.6, 12.4, 19.6, 28.6, 45.1, 65.7 which are practically in complete agreement with the values x_{II} calculated from equation (II). Hence the effect we are considering can be allowed for accurately by a very small modification in the assumed value of n , and a rather larger, but still small modification in the assumed value of K . In the actual fitting of an equation to a set of observations, the n and K arrived at are not, of course the true values, but already modified to allow for this effect. For the dissociation curve at constant cH the value of n is some 2 p.c. greater, and of K some 7 p.c. smaller, than the values actually found from a set of observations.

Whether, with solutions of hæmoglobin, the effect is as small as it has been shown above to be in the case of whole blood, we have not yet the means to decide. It is possible that salt-free, or buffer-free, hæmoglobin solution may have its cH considerably more affected than whole blood, by saturation with oxygen: if so, a much greater distortion may result; for example, if the cH were trebled by complete saturation, the apparent n would be reduced from 2.2 to 1.76. Such an effect might account for some of the anomalies observed with hæmoglobin solutions. At present, however, our lack of information allows us to do no more than state the problem.

APPENDIX II.

The mechanism of the $v\text{CO}_2$ — $c\text{H}$ relation. By A. V. HILL.

Referring to Fig. 3 in the preceding paper, suppose that we add bicarbonate to blood *isohydrically*, *i.e.* keeping the $c\text{H}$ constant while so doing by increasing the CO_2 -pressure in the same proportion as the total combined CO_2 . Then the *total* CO_2 in the blood will be increased (a) by the amount present in the bicarbonate added, and (b) by the extra amount dissolved under the increased CO_2 -pressure. The latter amount can be shown by calculation to be very small compared with the former, so that the addition of bicarbonate to blood increases the $v\text{CO}_2$ by practically a constant amount at all values of $c\text{H}$: it merely raises the $v\text{CO}_2$ — $c\text{H}$ line parallel to itself. Hence the degree to which the blood is buffered, as measured by the *slope* of the line in Fig. 3, is unaffected by the addition of bicarbonate to it. Adding bicarbonate to blood *in vitro* merely changes the CO_2 -pressure for a given $c\text{H}$: hence the immediate effect of adding bicarbonate to the blood in the body is merely to change the CO_2 -pressure in the alveolar air, without changing the slope of the $v\text{CO}_2$ — $c\text{H}$ line. Expressed in terms of equation (III) in the preceding paper, the addition of bicarbonate merely increases c without altering b . Hence the variations in c between different individuals may be attributed simply to variations in the amount of bicarbonate present in the blood.

If this be so we must look for variations in b , *i.e.* in the slope of the line, in the amount and quality of the contents of the red blood corpuscles. The $v\text{CO}_2$ — $c\text{H}$ line is far less steep for plasma than for blood, which is natural since the red cells contain the major portion of the phosphates and the whole of the hæmoglobin, which alone can provide the free base to combine with added CO_2 . Hence the slope of the line, and b , depend upon the quantity of phosphates and hæmoglobin present in the blood. In anæmic conditions associated with a low hæmoglobin content we should expect to find a small slope to the curve, with associated dyspnœa on exertion. Conversely acclimatisation to high altitudes, which leads to an increase in the amount of hæmoglobin in the blood, should produce a steeper curve, and so lead to a disappearance of the dyspnœa originally caused by exercise at low oxygen pressures.

STUDIES ON THE PHYSIOLOGY OF CAPILLARIES.

III. The innervation of the blood vessels in the hind legs of the frog. By A. KROGH, G. A. HARROP AND P. BRANDT REHBERG.

(From the Laboratory of Zoophysiology, Copenhagen University.)

THE investigations recorded in the present paper have arisen out of the study of the local reactions of the cutaneous vessels by one of us⁽¹⁾ and were undertaken primarily with a view to elucidate the mechanism of these reactions. As the experiments progressed it became necessary to modify and enlarge the scope of the research, and to repeat from new points of view experiments already done. The experiments were begun in January, 1920, by Krogh, carried on by Harrop, whose observations were partly checked by Dr v. Liebermann, and concluded by Rehberg who also repeated with an improved technique many of the earlier experiments.

We propose to deal:

1. With the effects on the vessels of stimulating the peripheral ends of the different fibres supplying the skin and the muscles of the hind legs.
2. With the effect of section and degeneration of these fibres upon the state of the vessels, the local reactions and the effects produced by stimulation in the sciatic of the remaining fibres.
3. With experiments on the effect of cocaine on the local reactions, and
4. With the existence or otherwise of true vascular reflexes causing changes in the calibre of vessels and in the circulation at and about the point stimulated.

Finally we shall attempt to synthesise our results into a picture of the innervation.

Methods. We have generally narcotised the frogs for the operations with urethane giving curari later when necessary. Operations, from which the animal is left to recover, require a number of precautions which were only gradually and, we fear, incompletely made out. *Rana esculenta* appears to stand the operations better than *R. temp.*, but for

observations on the web we had to use *R. temp.* Quite small frogs of 5-15 gm. weight were found to be the most suitable. The operations must be performed as far as possible under aseptic conditions and with the least possible damage to muscles and other tissues. Touching with adrenaline 1:1000 proved very useful to prevent excessive bleeding. We have filled up the defects produced in the vertebral column with soft, sterile paraffin wax and believe this procedure to be beneficial. Muscle sutures are to be avoided as far as possible, but the skin must be very carefully sutured and it is important to avoid any contact of the external surface with the internal or with the wound. The operated frogs were kept isolated in moist chambers with a little water. The water was changed frequently so as to remove the urethane by the end of 24 hours at least. Those which recovered (about 40 p.c.) were transferred after about a week to suitable terraria where they had access to water and were regularly fed. Several, especially among the smallest, gained considerably in weight during the following months. By the frequent examination of the web on these operated frogs the use of anaesthetics was avoided as far as possible. The frog, except the leg to be examined, was in these cases put into a small box and immobilised with wet cotton wool.

To stimulate the nerves we have used single induction shocks repeated at the rate of about one per second, tetanisations at a rate of 5-10 per sec. and in several cases current from a small dynamo giving variations of a type like $-o-o-o-o$, the frequency of which could be varied from 10 to 30 per sec. In some cases we have stimulated mechanically by pinching. In stimulating electrically sympathetic ganglia or the posterior roots of spinal nerves we have been able to work on animals which were narcotised only, since we could easily avoid the production of muscular movements and the absence of such served as a useful control, but in stimulating anterior roots we had of course to curarise. In such cases we have sometimes given both urethane and curari. The reactions obtained have been studied exclusively by microscopic examination, in most cases with a binocular microscope and a magnification of 40-60, but in the later stages of the investigation and where the reactions of capillaries were specially concerned usually with an ordinary microscope and magnifications of 100-120.

We have also made some trials with plethysmographic methods, but we find that the interpretation of the results is so difficult and generally so uncertain that the direct visual method is at the present stage to be preferred.

Stimulation of peripheral nerve fibres Stimulation of the sympathetic ganglia nos 8 to 10 according to Gaupp's nomenclature¹ gives constantly a contraction, generally very pronounced, of the arteries and capillaries in the web, both when the nerves behind them are left intact and when they are divided. Induction currents appear to be the most suitable stimulus, but the effect of the dynamo current is distinct also. After a latent period of 10-20 secs the stimulation produces constriction often to obliteration of the arteries². It is worthy of note that all the arteries are affected including those branches which do not respond to adrenaline. The capillaries become constricted generally 5-10 secs after the contraction of the arteries, but usually the contraction is not nearly complete, thus, a capillary of 15μ diameter contracted to 13μ , another of 17.5μ to 11μ . The contracted vessels rapidly relax again when the stimulus ceases and the reaction can be provoked several times in succession with intervals of about a minute. In some cases the effect is uniform, as far as can be judged, over the whole field, but in others some of the capillaries appear to escape stimulation. This is probably not due to any lack of sympathetic innervation but to the obvious difficulty of stimulating with sufficient intensity all the fibres or cells at the level of the ganglia. When the cut sciatic nerve is stimulated by induction shocks the effect is essentially the same as that of stimulating the sympathetic ganglia alone.

When a capillary is watched with a magnification of 200 while the sympathetic is stimulated it can be observed that the contraction starts from certain definite points along its course and spreads from these in both directions. At the same time a curious reaction appears to take place in the surrounding tissue which appears to become less transparent. While this latter phenomenon must be reserved for further study the meaning of the first observation will be fully explained in a paper by Vimtrup on the contractile elements in the capillary wall which is to be published shortly.

Stimulation of the sympathetic produces contraction also of muscle arteries and capillaries. In some cases the arterial effect only has been observed, bringing the blood flow in the capillaries to a standstill without producing any measurable contraction, in other cases several open

¹ Gaupp, *Anat d Frosches*, II p 212, 1899

² The latent periods given are approximate and have only some relative significance. The absolute time of latency depends upon temperature and is probably subject also to other influences. The effect of sympathetic stimulation upon arteries is of course well known. See the paper by Langlois (2) in which the literature is discussed.

capillaries have disappeared from view by contraction while others have remained open but with a diminished diameter as shown by the following measurements:

Diameter in μ	{before stimulation	11	11	13	15	17.5	17
	{during stimulation	8	8	8.5	11	11	10

Since a fall of arterial blood pressure even to 0 causes only the very slightest diminution in diameter of the capillaries there can be no doubt that the influence upon them of the sympathetic stimulus is direct.

Electrical stimulation of the posterior roots of the 9th and 10th spinal nerves produces distinct dilation of the web arteries as shown by the following examples:

Before stimulation	26	17	44	44	30	17 μ
During stimulation	44	30	48	61	43	30 μ

These changes occur after a latency of 15–40 secs. The vessels contract slowly when the stimulus ceases and the reaction can be repeated a few times only. In a few cases it has been noted that the peripheral arterial branches which do not respond to adrenaline are especially sensitive to the stimuli from the posterior roots and may during stimulation become wider than the stems from which they spring. Some capillaries also become dilated but a considerable number of these vessels do not respond to any stimulus from the posterior roots. In several cases we have like Doi(3) found it convenient to apply a small dose of acetyl-choline to the web to insure the filling of any capillaries the tonus of which might relax during the subsequent stimulation of the posterior roots. This procedure abolishes also the source of possible error which the dilatation of arteries and arterioles might otherwise constitute. The arteries dilated by acetyl-choline do not dilate further under the stimulation and the dilatation observed in capillaries cannot therefore be due to any increase in the blood pressure. Those capillaries which do dilate under the stimulus from the posterior roots often do so to a very considerable degree as shown by the following measurements:

Before stimulation	0	9	13 μ
During stimulation	8	15	17 μ

As shown by Doi(3) and confirmed by us mechanical stimulation of posterior roots will also produce dilatation of web capillaries, but we feel sure that in any case only a limited (though large) number of these vessels can be influenced through posterior root fibres. Mechanical stimulation by pinching of the sciatic nerve produces dilatation of the skin and web vessels as found by Bayliss in the case of mammals.

In muscles the effects of stimulating the posterior root fibres are not very marked, though in several cases a slight increase in the diameter of capillaries and the opening of a few new capillaries has been noticed. The effect on arteries appears generally to be very slight or even absent, but in a single case marked dilatation occurred.

In some experiments we have urethanised and curarised the frogs, extirpated the 8th-10th sympathetic ganglia and stimulated the 9th and 10th anterior roots with induction or dynamo currents. No trace of muscular contraction can be observed by the microscope, but after a latency of 10-20 secs some widening of arterioles takes place and there seems to be a slight opening up and dilatation of capillaries. We have never been able to imitate by stimulation of nerves the great increase in the number of open capillaries and in the current of blood through them which accompany actual contraction of the muscles. In the web no effect has been observed from stimulation of the anterior roots after extirpation of the sympathetic ganglia.

In Part II(1) it was shown that weak mechanical stimulation of one point in a web artery can produce dilatation of the artery over a length of one or more mm after a latency of about 15 secs while a strong stimulus produces contraction over a similar distance. In the light of the results given above these reactions must be taken to be due to stimulation of posterior root fibres and sympathetic fibres respectively, but more precise information regarding the mechanisms of these local reactions is obtained by the following experiments.

The effects of section and degeneration of nerve fibres. In one case the conduction of nervous impulses along the sciatic was temporarily blocked by freezing the nerve. the local arterial and capillary reactions remained perfectly undisturbed. In numerous other cases the sciatic nerve or certain of its roots were cut through, but apart from the resulting changes in arterial and capillary tone, presently to be described, the local reactions were unaffected. The whole of the reaction mechanisms must therefore be local and we must have to do either with axon reflexes or with short reflex arcs over local ganglion cells.

The vascular tone in the web has in our experiments never been affected by section of posterior or anterior¹ nerve roots or by removal of the 9th and 10th spinal ganglia, but removal of the sympathetic

¹ We cannot confirm the observations of Lapinsky(4) giving dilatation of blood vessels in the web after section of the anterior roots of the sciatic. Since, according to Langley(2) these roots do not carry preganglionic sympathetic fibres, the effects observed by Lapinsky are difficult to understand.

ganglia or section of the sciatic usually produces relaxation of arteries and capillaries. Sometimes this relaxation is observed at once, but more often it develops slowly and becomes pronounced after a few hours. The arteries usually regain their normal tone after a few days and in some cases appear not to lose it at all, but the capillaries often remain dilated for a long period extending even to 100 days. Very often however the arteries in the web of the operated side are found in a dilated state while the arteries of the normal side are narrow. The tone which develops after cutting off sympathetic influences appears to be very variable and the arteries have only a slight power of resistance against dilator influences.

The local arterial reactions have been followed in a number of operated frogs. It will be convenient to describe a few examples in some detail before proceeding to summarise the results.

In a small male frog, the right sciatic of which had been cut (with resection of about 1 cm.) on March 22nd, the examination on the 12th day showed contracted arteries and dilated capillaries (the left web was normal). The contraction reaction on strong mechanical stimulation of arteries was distinct, but in spite of the contracted state of the arteries the attempt to obtain dilatation failed. On the 19th day both arteries and capillaries were of normal appearance. The arteries were now hypersensitive, showing dilatation upon the slightest touch and contraction after stimuli which would normally produce dilatation. The capillaries reacted normally to mechanical stimulation, showing dilatation after weak, contraction after strong, stimuli. On the 28th day a number of abnormal reactions were observed after strong mechanical stimulation; the arteries sometimes showing contraction in the place stimulated and dilatation over a considerable distance in both directions, in other cases dilatation only, and in others again sharply localised contractions without any dilatation. The capillary reactions were still normal and if anything perhaps extended over a larger area than normally. When tested on the 42nd and 53rd day no dilatation of the arteries in the right web could be obtained by mechanical stimulation, though the arteries reacted promptly to acetyl-choline, but on the 60th day the dilatation reaction reappeared and remained distinct afterwards, while the contraction reaction became very indistinct at about this time though probably never completely absent until the observations were discontinued on the 123rd day. On some occasions a sharply local contraction appearing without any measurable period of latency could be elicited on most of the arteries, while a more or less normal reaction

over a short distance was obtained on a few. On the 95th day the capillary reactions to mechanical stimulation were tested. Dilatation only could be observed.

In four similar experiments the dilatation reaction was absent or very doubtful for a variable period, but reappeared in the two cases in which the animal lived long enough, after 100 to 150 days.

On a female frog of 20 gm. weight operated on April 2nd the spinal ganglia 9 and 10 were removed on the right side. The local reactions remained nearly normal for 50 days, but later the dilatation reaction became distinctly weaker and reduced in extent and failed to appear in several experiments between the 50th and 190th days. After 230 days it was, however, quite distinct, and remained so. The contraction reaction was normal throughout. In another similar experiment begun in June and lasting 140 days the dilatation was very slight on the 60th day, could not be elicited on the 101st, and reappeared on the 140th. Cutting of the anterior and posterior spinal roots 9 and 10 (one case) and degeneration for 145 days had no effect on the local reactions—as was to be expected.

Removal of the sympathetic ganglia 8 to 10 on one side (three cases) only diminished the contraction reactions to some extent and for a somewhat variable period between the 50th and 120th day. In one case which was only examined once after 77 days no contraction of arteries could be elicited. As mentioned above, the web arteries of these frogs were often strongly dilated and on such vessels it is very difficult and sometimes impossible to elicit the dilation reaction. It was observed however whenever the state of the vessels was suitable, and there is no reason to believe that it is in any way affected by removal of the sympathetic ganglia.

The operations were controlled in all cases where that was possible by examination of the sensitivity and reflex movements of the corresponding leg and in most of the cases by post-mortem inspection of the nerves. In one of the cases of resection of the sciatic a certain amount of regeneration had taken place after about 180 days, but the nerve was evidently abnormal and did not appear to function. In the cases in which the ganglion cells themselves had been removed regeneration was obviously impossible.

The results of these degeneration experiments are fairly consistent, but they differ greatly from those which we had to expect on the basis of current views on innervation. According to the nerve stimulation and nerve section experiments we confidently assumed that we had to

do with two distinct axon reflexes, one along sympathetic fibrils causing contraction and one along posterior root fibrils causing dilatation. We expected therefore that degeneration of sympathetic or posterior root fibres would abolish the corresponding local reactions. While it is quite clear that the local reactions are temporarily inhibited by degeneration of the nerve fibres through the fibrillar branches of which we believe them to be conducted, it is equally certain that they are not completely abolished and return practically to normal sensitivity and intensity though no regeneration of the fibres belonging to the extirpated sympathetic or spinal ganglia can possibly take place. It will require experimentation along new lines, combined with histological investigation, to clear up this paradox, but a tentative explanation will be put forward at the end of this paper.

On several of the operated frogs the sciatic was exposed and stimulated electrically by induction and dynamo current before the animals were killed. In one case the right spinal roots 9 and 10 had been cut through and the right sympathetic ganglia removed 67 days earlier, so that the only active fibres which could be present must be posterior root fibres from the 9th and 10th spinal ganglia. The stimulation produced dilatation of web arteries and of arteries in a muscle. In the muscle a slight widening of capillaries was observed and a corresponding widening of web capillaries had undoubtedly taken place but is not recorded in the protocol. In another frog the spinal and sympathetic ganglia had been removed 77 days. In this case no effect on the web vessels could be detected when the sciatic was stimulated. The examination of the muscles, which would have been of special importance, was unfortunately omitted. In several cases in which the sympathetic ganglia only had been removed stimulation of the sciatic caused dilatation of vessels both in muscles and in the web. These results confirm the stimulation experiments described above.

Cocaine experiments. A rather large number of experiments have been made to test the effect of cocaine and allied substances in the hope of finding a definite difference between their influence upon the contraction and dilatation reaction respectively. The application of 1-2 p.c. chloride of cocaine or novocaine in large drops or by means of reaction basins generally shows very little influence upon the reactions during the first hour or half-hour, but later the dilatation reaction usually becomes diminished or abolished while the contraction reaction is sometimes unchanged, sometimes more or less diminished. Experiments on animals which had not been narcotised show clearly that cocaine must

be absorbed very slowly through the skin since reflex movements can be induced by pinching of toes after one hour's bathing in 2 p.c. cocaine. When 2 p.c. cocaine or 1 p.c. novocaine is injected into the web by a micropipette with a very fine point the arteries after a very transitory contraction become more or less dilated and after a few minutes do not react at all to weak mechanical stimulation while strong stimuli sometimes produce a local dilatation and in other cases remain without any visible effect. Nothing definite can be concluded therefore from our cocaine experiments.

Experiments on vascular reflexes. All the vascular reactions so far described as resulting from local stimulation of the tongue or skin of the frog depend on local mechanisms—nervous and others—but not upon true reflexes, and the problem very naturally suggests itself. Are the local mechanisms the only ones existing in the frog for the production of vascular reactions about a stimulated skin area, and are the vascular reflexes which are so conspicuous in the skin of mammals to be considered as belonging to a higher stage in the development of the central nervous system?

We have found that chemical stimulation may cause dilatation of arteries and capillaries over a large area of the web even beyond the space between two toes where the stimulus is applied. Iodine may show this effect, but the most powerful stimulant appears to be nitrate of silver, which has the advantage that the direct corroding effect spreads over a very limited and well defined area only. When a small crystal of silver nitrate is placed on the web all the arteries between the two toes and often a large number between neighbouring toes will dilate after a latency of about 10 secs. A few seconds later a large number of capillaries will also show some dilatation which is certainly in the main independent of the increase in pressure and blood flow resulting from the widening of arteries. The arteries will sometimes show a rapid succession of changes in diameter, while the capillaries remain dilated for a long time.

Example. Crystal placed near one of the toes. Observation of artery from opposite side. Initial diameter 30μ . After 10 secs. 35μ and thereupon 26–44, 26–39.

This response (which in appearance resembles closely the reflex erythema produced by mechanical, chemical or thermal stimulation of the human skin) depends upon a local mechanism, since it persists after section of the sciatic. In three frogs, the sciatics were cut on Sept. 26th and the reactions were examined on Oct. 8th at a period when the

THE LOCALISATION OF EXCRETION IN THE URINIFEROUS TUBULE. BY J. M. O'CONNOR AND E. J. CONWAY.

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THE theories of secretion of urine are so well known, and so fully discussed⁽¹⁾ that a consideration of them, except in so far as they form a basis from which this investigation starts, is unnecessary. The two radical theories of Ludwig and of Bowman and their various outgrowths differ fundamentally in this, that according to the Ludwig theory the entire excreta of the kidney must pass into the tubule through the glomerulus, whereas according to the other class of theories particular substances can join the urine in its course through the convoluted tubes.

If we apply the Ludwig view to any particular substance we should expect that on an injection of that substance into the blood stream, the urine emerging from the lower end of the ureter would show an increased concentration only when the previous contents of the ureter, pelvis, collecting tubes and uriniferous tubules had been expelled. If on the other hand the substance reached the urine even in part through the second convoluted tubule an increased concentration would show itself when the contents of the system from the junctional tubule downwards had been expelled.

An approximation to the value of these quantities in the rabbit was obtained as follows: The diameter of the ureter of a living rabbit was measured by a low-power microscope as 1 mm. The thickness of the wall as found afterwards from sections was about 0.2 mm., the radius of the bore was consequently 0.3 mm. The length of the ureter in the rabbit is 10 to 12 cms. The capacity of the ureter is consequently about 30 c.mms. The capacity of the pelvis within the kidney to beyond the tip of the pyramid was calculated from its average area in serial sections of a kidney taken from a block of known thickness at about 6 c.mms. Seeing no prospect of obtaining data for calculating the volume of the collecting tubules and ducts without greater labour than it seemed worth, we have not attempted it. The volume of the uriniferous tubule in the rabbit was obtained from the average diameter, the average length and the number. The average diameter of the cavity of the convoluted tubes as obtained from a series of sections fixed with corrosive sublimate and embedded in celloidin was 12μ . The average diameter of the U tubes in the same kidney was 12.2μ . The length of the uriniferous tubule may be taken at 20 mms. (Huber⁽²⁾). The number was obtained by cutting an entire rabbit's kidney (from Exp. 5, Table II) in sections of 25μ and counting the glomeruli in every fourth section. As the glomeruli were approximately 100μ in diameter the total count, 55,000, is approximately

the total number of tubules in the kidney. The contents of the uriniferous tubules is consequently in this case about 120 c mms. The uriniferous tubules are it is true not all of the same length and do not all enter the collecting ducts at the same level, but such departures from the average would scarcely produce significant confusion.

These figures can scarcely be regarded as more than illustrative, the ureter is very distensible and slight obstruction to the flow would alter its volume considerably, and we know that the capacity of the uriniferous tubules varies with the activity of the kidney (3). Using them merely as an example one would expect that if the urine coming from the lower end of the ureter were collected in drops of 20 c mms a substance excreted by the second convoluted tubule would appear, or appear in increased quantity, in the third or fourth drop after the injected substance reached the kidney, but a substance excreted by the glomerulus only would not show itself until the eighth or ninth drop.

The time the injected material reaches the kidney can be taken as 7 seconds after the injection (4). As the convoluted tubules lie close around the glomerulus to which they belong the arrival of the injection at the glomerulus and at the convoluted tubule may be taken as simultaneous. Obviously the method of enquiry outlined is of general application requiring merely methods of analysis sufficiently delicate to permit approximate analysis of drops of the urine of the order of 20 c mms.

We have applied the plan to three substances, chlorides, uric acid, iodides. All the experiments were done on rabbits. The anæsthetic used was urethane by the mouth in a dose almost invariably of $1\frac{1}{2}$ grams pro kilo. An injection cannula was fitted into the jugular vein, and as short a cannula as practicable introduced into one ureter, generally the right. The ureter cannula had been bent at right angles, and the drops of urine as they fell off its end were collected in numbered depressions made in small slabs of paraffin wax. The fall of each drop was signalled on to a drum with time record. When a sufficient number of drops had been taken the substance to be studied was injected into the jugular vein, the time of injection being indicated on the drum. As the substances were usually injected in a large quantity of water the rate of the falling of the drops generally showed an immediate acceleration. After collecting sufficient drops, a number were allowed to fall on to a dry watch glass, the increase in weight of which was used to calculate their volume, the specific gravity being taken as unity. Finally the volume of the ureter cannula was obtained by weighing it empty and filled with mercury. In the quantities in the tables allowance has been made for this.

Chlorides. The method used for estimating the chlorides was a precipitation of chloride in acid solution by excess of silver nitrate and titrating the excess with standard iodide using starch and nitrite as an indicator(5). Each drop of urine was transferred from its paraffin slab into a miniature test tube, a drop of strong nitric acid $\frac{1}{2}$ c.c. N/30 AgNO₃ added. The test-tube was filled up to a mark indicating 1 c.c. The tubes were allowed to stand in the dark over night, then after a few turns of a hand centrifuge to bring down adherent granules of precipitate $\frac{1}{2}$ c.c. was drawn off into a porcelain dish and titrated after the addition of a few drops of a starch and nitrite with N/1000 iodide. The end point was in our experience very sharp. The results are conventionally expressed

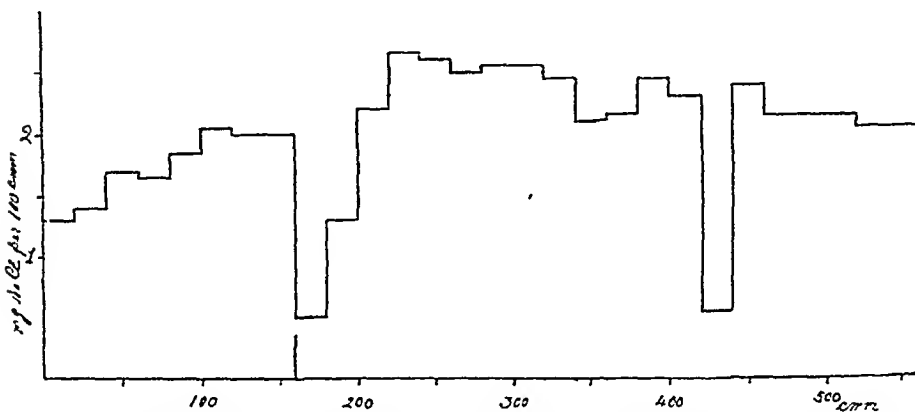


Fig. 1. Experiment with sodium chloride. Abscissa, in this and other Figs. = quantity of urine passed in c.m.m.s. At the vertical line ureter clamped for 10 mins. Average rate of flow previous to obstruction, 10 c.m.m.s. in 57 secs.; of first 10 drops after removal of obstruction, 10 c.m.m.s. in 4 secs.; of second 10 drops after removal of obstruction, 10 c.m.m.s. in 16 secs. Size of drops, 20 c.m.m.s. Volume of cannula, 14 c.m.m.s.

as sodium chloride. Control experiments gave fairly consistent results though the p.c. of chloride was invariably too low. As, however, we desired merely comparative values this was immaterial.

Realising that histological estimates of the volume of urine in the kidney needed confirmation we endeavoured to obtain it in the following fashion: Whatever may be happening at any time within the kidney it is to be expected that the urine will be altered by a delay in the flow; and that the application of a clamp to the ureter for a period would cause an alteration in the percentage of chlorides in the urine filling the kidney during the period. Consequently on removal of the clip the quantity of urine showing alteration would be a measure of the capacity of the kidney. This measurement would it is true be above the normal

owing to accumulation of the urine. A number of experiments on these lines were tried. Attention may be directed to one which gave very distinct results (cf. Fig. 1). It will be seen that from this experiment the capacity of kidney and ureter may be estimated at 270 c.c.mms.

Experiments on the excretion of chlorides after injection of a solution of sodium chloride were done chiefly with a .9 p.c. solution. One experiment was done with 2 p.c. As the flow of urine after the injection is so rapid as to suggest (on grounds discussed later) a risk of confusion, gum was in some cases added to the injection fluid with a view to decreasing the diuresis.

The results obtained are collected in Table I. The point of increase in chloride is determined by the first drop which could be taken as indicating a definite increase. The experiments fall into two classes. In Exps. 1, 3, 4 the increase appeared early, at a point which may be taken as indicating excretion by the second convoluted tubules. In the others the rise occurred after the expulsion of a larger quantity of urine suggesting that excretion in these cases occurred at a higher level in the tubule. As the percentage of sodium chloride was usually rather high and the increase in concentration on injection of a chloride solution not usually very pronounced, chloride did not seem to lend itself readily to the method and was not further tested.

Uric acid. As uric acid is a substance which even defenders of the Ludwig theory seem tempted to regard as a special case, we directed attention particularly to it.

Uric acid was injected in a solution in phosphate. Its amount in the urine we estimated by the colour produced with Folin's re-agent (c). The contents of the depression on the paraffin slab were washed with a fine pipette into the tube of a Gower's hæmoglobinometer. To this a few drops of Folin's reagent and of strong sodium carbonate solution were added. The resulting colour was diluted until it matched the standard. This reaction can of course be produced by other substances occurring in urine as well as by uric acid, but it is legitimate to assume that an increase in the reaction on injection of uric acid is due to an increase of uric acid in the urine. The standard solution for comparison gave some difficulty. At first a standard uric acid solution was used but the frequent renewal made necessary by the rapid fading of the colour caused considerable delay. Eventually a solution of copper sulphate and ammonia suitably diluted was found to give a close approximation to the correct colour. This was made up in a hæmoglobinometer tube to match the colour produced by Folin's reagent with

TABLE I. Sodium chloride.

Exp.	Body wt.	Fluid injected			Secr. time (secs.) of 10 c.mms.		C.mms. urine before inc. in % NaCl	Time (secs.) of inc. after inject.	% NaCl	
		c.c.	NaCl %	Gum %	before inject.	after inject. (10 drops)			average before	max. after
1	1.5	45	0.9	—	90	7	26 or 48	100	0.1	2.0
2	2.2	30	0.9	—	40	6	121	80	1.9	2.6
3	1.7	10	2.0	—	20	4	48	49	1.9	2.3
4	2.2	25	0.9	8	23	13	49	170	1.1	1.7
5	2.0	30	0.9	6	30	15	124	106	1.4	2.1

TABLE II. Uric acid.

	Body wt.	Fluid injected					C.mms. urine before inc. in % uric acid			
		c.c.	UA %							
1	2.0	40	0.02	—	33	16	26	168	—	—
2	2.7	40	0.02	—	23	17	63	174	—	—
3	1.9	10	0.1	—	64	40	27	151	—	—
4	1.9	30	0.02	—	10	9	112	151	—	—
5	1.5	32	0.02	—	154	38	48	378	—	—
6	2.5	30	0.02	—	30	25	28	283	—	—

TABLE III. Sodium iodide.

	Body wt.	Fluid injected					C.mms. before inc. in % NaI			
		c.c.	NaI %							
1	1.7	20	0.9	—	26	10	142	158	—	—
2	2.0	7	0.9	—	13	5	86 { 182 ¹	78 { 110 ¹	—	—
3	1.9	10	0.9	—	5	6	175	134	—	—
4	1.4	22	0.9	—	12	6	116	111	—	—
5	2.5	25	0.35	—	15	19	186	333	—	—
6	1.3	20	1.7	—	10	3	214	51	—	—

TABLE IV. Uric acid and sodium iodide.

	Body wt.	Fluid injected					C.mms. before inc. inj.				C.mms. between UA of NaI on reappearance
		c.c.	UA %	NaI %			UA	NaI	UA	NaI	
1	2.5	25	0.018	0.35	35	28	90	146	403	640	92
2	2.6	25	0.018	0.35	26	23	56	131	190	367	—
3	2.0	5	0.1	0.35	22	?	140	168	?	?	28
4	1.6	10	0.02	0.8	20	12	72	30	87	56	—
5	2.5	15	0.014	0.6	7	11	212	316	308	442	—

¹ The iodide having appeared at the figure given above, there was at this point a large and lasting increase.

a fresh standard uric acid solution in another similar tube. The standard commonly used matched .008 mg. uric acid diluted to .8 c.c. The results of the experiments are summarised in Table II (Exp. 1 illustrated in upper graph, Fig. 2). From the table it will be seen that uric acid in the majority of the cases comes out at a point suggesting excretion through the tubules. It is clear that if the calculations given previously are at all like the true relations, the uric acid coming out after 50 c.mms. of urine have been expelled must be excreted through the second convoluted tubule.

That the volume of the ureter and pelvis is in any ordinary case less than 30 c mms is very improbable but it may be thought that the uriniferous tubule proper is sometimes much narrower than stated, and that an excretion through the glomerulus might trickle down through such contracted tubules and appear at much the same point as would a substance excreted by the tubule. That this argument is, however, improbable is shown by the fact that similar results were obtained in cases where the urine before the injection was excreted at different rates in which consequently the tubules would be expected to be distended to different degrees. That the quantity expelled before the rise in the percentage of uric acid is in some cases less than what would be expected is probably due to diffusion or to mixture in the ureter due to peristaltic waves. That diffusion in the tubules themselves could cause any confusion is extremely improbable.

With regard to mixture in the ureter it was sometimes noticed that a drop of urine was expelled by a peristaltic wave rapidly after a series of drops which had fallen in regular sequence. A third drop was never expelled by such a wave. This gives support to our calculation that the ureter does not usually contain more than two drops of 20 c mms as if it did, peristalsis would force out a gush of more than two drops. Mixture in the ureter would consequently not cause a larger error than one drop of 20 c mms. Another cause of the quantity expelled before the rise in excretion appearing too small is that an increased excretion occurring towards the end of the drop will be regarded as occurring towards the beginning of the drop. This error could scarcely amount to more than 15 c mms. Consequently at the utmost the figures given may be 35 c mms. too small.

One result (Exp. 1, Table II) is out of keeping with the idea of excretion of uric acid by the convoluted tubules. It will be noticed that in this case the excretion of urine before the injection was very rapid. It might be held that although the arrival of the injected substance at the convoluted tubule is calculable we know nothing about the time required for it to pass through the wall. This time may be so long that at a rapid rate of flow an appreciable quantity of urine might have passed down the tubule from the glomerulus before the increased uric acid could have passed through the wall of the tubule. Against this point it may be urged that in that case one would expect increasing rapidity of flow to be associated in the series of experiments with a gradual rise in the quantity of urine expelled before the increase in uric acid appears. This is not the case. The evidence then points to uric acid being usually excreted by the second convoluted tubule but sometimes only by higher portions of the tubule probably the glomerulus.

Iodides Titration methods for the small quantities of iodide obtainable from a drop of urine did not prove satisfactory and even freeing

the iodine and extracting with a drop of chloroform did not give sufficient colour to be used colorimetrically. We were consequently compelled to fall back on the depths of colour produced by the freed iodine on starch paste. For this purpose the drops were transferred to a hæmoglobinometer tube, sodium nitrite and nitric acid and excess of starch were added and

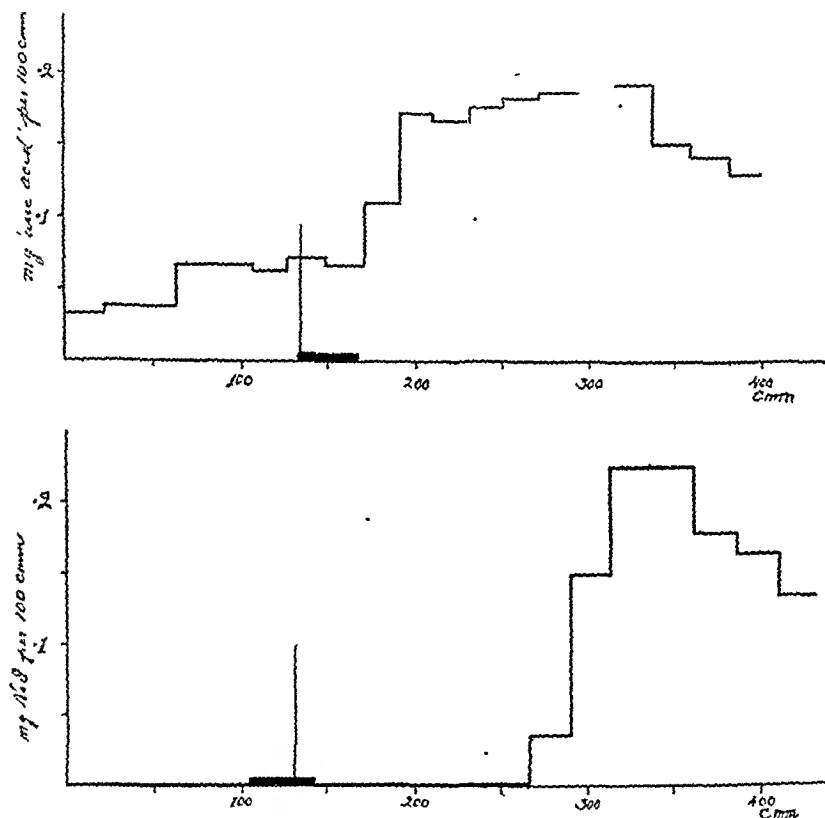


Fig. 2. Upper curve, experiment with uric acid. The black rectangle on the abscissa indicates the approximate period taken by the injection. The vertical line the approximate first arrival of the injected fluid at the kidney. Size of drop, 21 c.mms. Volume of cannula, 10 c.mms.

Lower curve, experiment with sodium iodide. Indications as in upper curve. Size of drop, 24 c.mms. Volume of cannula, 10 c.mms.

the solution was then diluted until the blue colour matched the standard. The standard of copper sulphate and ammonia used for the uric acid estimations proved suitable for the starch-iodine colour also.

The results of the experiments with the iodides are summarised in Table III. It will be noticed that, with the doubtful exception of Exp. 2,

they all point strongly to the view that iodide is excreted high up in the tubule, probably in the glomerulus. Fig. 2, lower graph, illustrates Exp. 1 of the series. Exp. 1 is selected for illustration as in it the conditions previous to the injection correspond most closely to the example of uric acid excretion illustrated in Fig. 2, upper graph.

It might be thought that the later excretion of iodide compared to uric acid might be due to slower excretion of iodide by the tubule than occurs in the case of uric acid. That this explanation, in itself most improbable, does not hold is indicated by the fact that the times are on the average shorter than in the uric acid experiments and seems definitely disproved by the experiment referred to on p. 199. A further possibility is the one already advanced in the anomalous uric acid experiment; that the iodide is in these cases excreted by the tubule but the flow of urine is so rapid that an appreciable quantity of urine has passed by the second convoluted tubule between the time the iodide reaches the blood around the convoluted tubule and the moment of arrival into the cavity of the tubule. The improbability of this explanation need not be reiterated.

Uric acid and iodide. The previous experiments have gone to show that in uric acid and in iodide we have two substances excreted at different levels of the uriniferous tubule. The evidence points to uric acid being excreted low down, probably in part in the second convoluted tubule, while the iodide is excreted at higher level, probably the glomerulus.

Additional evidence for this was obtained by injecting uric acid and iodide simultaneously. In some of these cases where the normal uric acid reaction was very marked it was found possible to take half of each drop, previously diluted in a hæmoglobinometer tube, for each estimation. In others we were compelled to determine each substance on separate drops which were taken alternately or otherwise as circumstances indicated. As every drop was in these cases not examined for both substances the quantity of urine expelled before the increase or appearance of the substance tends to be exaggerated. The injections were given in small amounts and rapidly, thus preventing extensive mixing of the injected material with the blood. In this way we were in some cases able to demonstrate a return of the uric acid to normal and a disappearance of iodide followed by a new increase in uric acid and a reappearance of iodide after a time on the return of the injected fluid in the course of the circulation.

The results obtained in these experiments are given in Table IV.

claimed, is less sure. On the question whether it is also excreted by the portions of the uriniferous tubule up to the iodide excreting region these experiments do not permit a judgment. The identification of the iodide excreting region with the glomerulus is also to some extent arbitrary. We claim for it merely probability. It need not be pointed out that our experiments give no basis for a decision on the nature of the excretory process in the glomerulus.

SUMMARY.

(1) It is suggested that as a substance excreted by the convoluted tubule would join the urine lower down in its course than if excreted through the glomerulus it would have to expel a smaller quantity of "dead space" urine than if excreted by the glomerulus.

(2) Approximate calculations as to the volume of these different "dead spaces" in the rabbit are made.

(3) Experiments on the intravenous injection of sodium chloride suggest that while sometimes excreted by the tubule it is in other cases excreted by the glomerulus alone.

(4) Experiments with uric acid go to show that it is excreted by the second convoluted tubule.

(5) Experiments with injections of sodium iodide show that iodide is generally excreted at a higher level, probably the glomerulus.

(6) Experiments in which uric acid and iodide were injected together confirm these views.

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GLUCOSE ABSORPTION IN THE RENAL TUBULES OF THE FROG. BY G. A. CLARK.

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OPINION is divided on the question of how the kidney prevents the passage into the urine of the glucose normally present in the blood. The modern theory assumes that the blood-sugar is present in the glomerular filtrate and is reabsorbed by the tubule epithelium, while according to Heidenhain the glomerular membrane is impermeable to glucose. Evidence in support of the latter view has been produced by Hamburger⁽¹⁾ who perfused the frog's kidney by way of the renal arteries with a modified Ringer's fluid containing glucose and found that a sugar-free urine was formed when the perfusing fluid contained .08 p.c. (and occasionally .12 p.c.) glucose. Because the glomeruli in the frog are supplied by the renal artery only, it was held that this retention of glucose was due to some function of the glomerular membrane; it was not fully appreciated that, under the conditions of experiment, the tubules were capable of their normal activity. In the frog the efferent vessel from each glomerulus opens into the venous spaces surrounding the tubules, so that the latter must be bathed by any fluid flowing through the renal artery. Hamburger also determined that when the perfusion contained .25 p.c. glucose or more, the kidney allowed the whole of this glucose to escape into the urine. This was attributed to a "sickening" effect on the glomerular membrane.

The experiments described below were undertaken to ascertain if the renal tubule played any part in preventing the blood-sugar from passing into the urine.

The method of perfusion employed was that described by Bainbridge, Collins and Menzies⁽²⁾. Winter frogs which had been kept in a semi-dark tank were used, and whose blood-sugar content was found to vary between .020 p.c. and .027 p.c.; these figures correspond with those determined by Bang⁽³⁾, whose micro-chemical method for the estimation of sugar was used in these experiments. The perfusing fluid, in which varying amounts of sugar were dissolved, had the following composition: NaCl .5 p.c., KCl .01 p.c., CaCl₂ .02 p.c., NaHCO₃ .285 p.c.

and reabsorbed by the tubule epithelium, but that this reabsorption cannot proceed under the conditions described. By substituting a venous perfusing fluid having the same concentration of glucose as the arterial, it was found that the kidney gradually regained the power of retaining glucose. Unfortunately it was not possible to maintain the secretion of urine long enough for the return of the normal "threshold," but usually after one hour the kidney could retain about .03 p.c. to .04 p.c.

In Exp. 11 (Table I) it is seen that the urine from the right kidney contained no glucose, while that from the left showed the same concentration as the arterial perfusion. This was due to an accident by which the right renal-portal vein was included in the ligature holding the ureteral cannula in position; the right kidney therefore was receiving no venous perfusion and, as would be expected, was capable of its normal activity.

Throughout the experiments described, the rate of formation of urine bore no relation to the sugar-content of the perfusing fluid. In the mammal, diuresis in glycosuria is due to the osmotic resistance offered to the absorption of water in the tubule by the glucose content of the glomerular filtrate(7); in the frog, however, no absorption of water occurs in the tubules, as can be seen from Table I, where the urine contains exactly the same percentage of sugar as the arterial perfusion. The absorption of water appears to be actually resisted, for the physical conditions invite the passage of water from the glomerular filtrate to the more concentrated fluid in the tubule capillaries. In only one experiment (not quoted) was the sugar content of the urine higher, but in this case the arterial perfusion pressure was less than twice the venous pressure, thus admitting the possibility of the venous perfusing fluid reaching the glomeruli. The tubule epithelium must therefore be regarded as an irreciprocal membrane, so far as glucose is concerned, of the same type as that formed by the epithelium of the small intestine.

Additional evidence of the function of the renal tubule to absorb glucose was furnished by experiments in which the tubule epithelium was poisoned by mercuric chloride or sodium arsenite. A .01 p.c. solution of the former was used and the latter was prepared by dissolving .5 gm. arsenious oxide in 100 c.c. of Na_2CO_3 solution. Both aorta and renal-portal vein were perfused with fluid containing less than .1 p.c. glucose. When the kidneys had been washed free from blood about 5 c.c. of the toxic solution were passed through the venous cannula. At the end of the experiment the renal-portal vein was perfused with dilute $(\text{NH}_4)_2\text{S}$ when the mercuric salt had been used and the kidneys examined in

serial sections; when sodium arsenite was employed, .5 p.c. CuSO_4 was passed through the venous cannula; with the arsenite this formed the insoluble "Scheele's green," which could be distinguished microscopically. Whenever evidence of the presence of the poison was found in the glomeruli, the experiment was discarded, but the only difference observed between those cases where the tubules alone were affected and those where the glomeruli showed a deposit was that in the latter the rate of formation of urine was slower, even when the perfusion flow was normal, although this last was usually reduced. In every case the urine contained the same percentage of glucose as the perfusing fluid.

SUMMARY AND CONCLUSIONS.

(1) The glomerular membrane is completely permeable to glucose, even when the latter is present in the blood below the "threshold value."

(2) The epithelium of the renal tubules has the power to absorb glucose from the glomerular filtrate up to the normal "threshold value." This absorption is possible until the capillaries surrounding the tubules contain glucose at a concentration of nine or ten times that normally present in the blood.

(3) In the absence of calcium the renal tubule is no longer capable of its normal activity.

(4) No concentration of the glomerular filtrate by absorption of water occurs in the frog.

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THE SECRETION OF SWEAT. Part II. The effect of vaso-constriction and of adrenaline. BY J. N. LANGLEY AND K. UYENO, M.D., *Tokio.*

(*From the Physiological Laboratory, Cambridge.*)

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IN Part I(1) it was mentioned by one of us that injection of 0.1-0.15 c.c. of Ringer's fluid into the pad of a cat's foot usually, but not always, causes more secretion of sweat than injection of adrenaline 0.01-1 p.c., and that adrenaline so injected causes a more or less long lasting depression in the response of the glands to pilocarpine. The explanation of the effect of adrenaline solution appeared to be that the fluid in which the adrenaline was dissolved caused the secretion, and that the vaso-constriction produced by adrenaline itself caused the decrease in response. The results however might be taken as showing that adrenaline has both a secretory and an inhibitory action on the gland cells, the inhibition being usually dominant. Since this theory would tend to bring the action of adrenaline on the sweat glands into line with its action on other tissues, we have compared in further experiments the effect of injecting Ringer's fluid with that of injecting adrenaline, and have investigated the effect of vaso-constriction. Considerable attention to detail is unfortunately required in order to arrive at a definite conclusion.

The experiments were made on cats of various ages. As a rule the animals were anæsthetised with C.E. mixture, but in order to make certain that the anæsthetic did not affect the results, some were made on animals decerebrated and only anæsthetised before decerebration and a few on "spinal" animals. The adrenaline and other solutions injected were usually warmed to body temperature, but in the few experiments in which the fluids were injected at room temperature the results were the same. The solutions were injected into the subcutaneous fat tissue,

usually into the pad from the front, occasionally also into one of the toes; the amount injected was usually 0.1–0.15 c.c., occasionally 0.2 c.c., a larger amount was only injected for special purposes. Since all four feet secrete, several observations can be made on any one animal. As a rule the cat was placed lying down on its side and unfastened. It was placed on a warmed surface, and the feet were wrapped in warm cloths. For quick recognition of the secreting parts, it is convenient to mark the right limbs and the inner surface of each. The secretory surfaces were examined with the aid of a lens whether the secretion was obvious to the eye or not.

The stock adrenaline solution was 0.1 p.c. Both Parke, Davis' and Burroughs and Wellcome's adrenaline chloride preparation were used; in each the adrenaline salt is in a faintly acid medium and contains either chloretone or chloroform; we have not noticed any distinct difference in their action. From these, solutions of .01 and .001 p.c. were made by diluting with cold Ringer's fluid. The amount injected was only warmed immediately before injection. A few experiments were made with crystallised adrenaline dissolved in a minimal amount of dilute hydrochloric acid, with results similar to those produced by the commercial preparations.

The secretion caused by fluids.

In the experiments referred to in Part I, Ringer's fluid caused secretion in a considerable majority of the experiments. The experiments were made in the late spring and the summer months, and chiefly on half-grown cats. We obtained a fairly similar result up to the end of January. During February and March the result was different. In 16 experiments in which Ringer's fluid or adrenaline or both were injected into the pads of two or more feet no secretion was obtained in 12; in some of these both sciatic nerves were cut. In two (both spinal cats) Ringer's fluid caused a slight secretion, in one it caused a trace. In one both Ringer's fluid and adrenaline caused a slight secretion, the former rather more than the latter. The difference in excitability was probably due, in part at any rate, to the animals having been older in the later experiments. In this and the succeeding section we refer, unless otherwise mentioned, to the experiments made up to the end of January.

The following fluids, in addition to Ringer's fluid and adrenaline .001 to .75 p.c. were found to be capable of causing secretion when injected into the pad of the foot. Adrenaline solution in which the adrenaline had been destroyed by warming it with a trace of alkali. distilled water,

Ringer's fluid with four times the normal percentage of salts, barium chloride $\cdot 1$ to 1 p.c. dissolved either in Ringer's fluid or in distilled water, 1 p.c. calcium chloride, amyl nitrite, 1 p.c. sodium nitrite, and pituitrin (Allen and Hanbury's preparation). The secretion when produced varies greatly in time of visible beginning, amount and duration. It may be obvious in a second or two, or not be visible to the eye for several minutes. With Ringer's fluid the secretion may last any time from five minutes to an hour. The secretion caused by adrenaline lasted usually 5 to 10 minutes, but in the experiments in Tables I and II in which $\cdot 01$ or $\cdot 001$ p.c. caused a moderate secretion it lasted 15 to 20 mins.

The number of experiments made with most solutions were insufficient to allow any strict comparison of their relative secretory effect. Amyl nitrite in one case caused a very copious secretion—helped no doubt by the increased blood flow—but with a certain decrease of excitability it had no effect, though pilocarpine still caused a moderate secretion. Hypertonic salt solution caused less secretion than Ringer's fluid with which it was compared. Barium chloride $0\cdot 1$ to 1 p.c. had about the same effect as Ringer's fluid, sometimes more sometimes less. Calcium chloride 1 p.c. in two experiments caused a slight secretion only, but in these barium chloride 1 p.c. also had only a slight effect. Pituitrin once caused no secretion, once a slight secretion, and once a free secretion. In experiments in March, pituitrin caused a slight secretion when Ringer's fluid did not (2 exps.); histamine $\cdot 1$ p.c. caused a slight secretion, 1 p.c. caused none; eserine $\cdot 5$ p.c. (1 exp. in each case) and ergotoxine phosphate $\cdot 1$ p.c. (2 exps.) caused none.

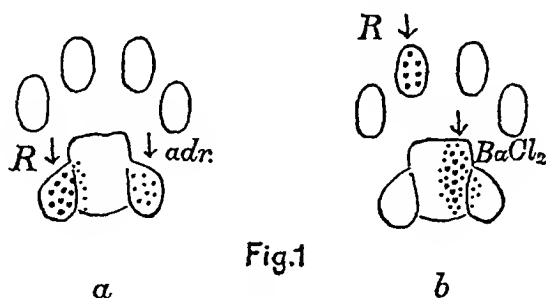


Fig. 1. Restriction of the secretion (when produced) to the injected area.

(a) Secretion was caused by injecting $0\cdot 1$ c.c. of Ringer's fluid into the lateral side pad of a foot and a minute later by injecting $0\cdot 1$ c.c. of $0\cdot 1$ p.c. adrenaline into the medial pad. The maximal secretion (about 5 minutes later) is shown diagrammatically.

(b) Secretion 5 minutes after injecting $0\cdot 1$ c.c. barium chloride 1 p.c. into the antero-lateral part of the mid pad, and $0\cdot 1$ p.c. Ringer's fluid into one toe.

The secretion produced by each of the fluids is more or less strictly confined to the injected area (cp. Fig. 1). If $0\cdot 1$ c.c. is injected into the middle of the pad (mid pad) no secretion is caused in the small lateral eminences (side pads) except it may be with nitrites. In no case does

injection into the pad cause secretion in the toes, or injection into one toe cause secretion in any other part of the foot.

When a secretion has been caused by injecting Ringer's fluid, a second injection in the same area causes usually less secretion and may cause none. The result depends upon the initial excitability of the glands and upon the degree of decrease of excitability in the interval, due to decreased circulation and cooling of the feet and to fatigue. But there is usually some decrease of response as the direct result of the injection (cp. p. 213). Since the secretion with all the fluids is local, observations on the effect of more than one fluid can be made on the same foot. But there is a limitation to this method, since some solutions, though only causing secretion in the injected area, depress the response to pilocarpine outside the injected area. This will be referred to later.

The secretory effects of all the fluids is prevented by previous injection of a small amount of atropine. The amount we have injected has varied from 1 to 5 mgms. of atropine sulphate. The suppression of the action of barium chloride is noteworthy, since on unstriated muscle, the point of action of barium chloride is held to be the general muscle substance and not the receptive substance. The result tends to show that BaCl_2 has no direct secretory action. The secretion caused by fluid is not due to mechanical stimulation of the nerve filaments by stretching them, since the injection of air does not, so far as we have seen, cause secretion, and since Ringer's fluid may cause secretion in denervated glands (cp. Part I, p. 118). The fluid must have a direct action on the gland cells. It would appear that the glands, if readily excitable, secrete at once when there is excess of fluid around them, whether the fluid is hypotonic or hypertonic.

Ringer's fluid, up to 100 c.c. injected intravenously at a time after pilocarpine when the secretion is slow causes a slight increase only. We have not tried this at the beginning of an experiment when the excitability is greater.

Relative amount of secretion caused by Ringer's fluid and by adrenaline.

In 11 experiments (including those on which Part I was based but omitting the three in which pilocarpine caused no secretion or only a trace) adrenaline was injected and not Ringer's fluid, it caused secretion in 6 (3 with .1 p.c. secretion slight; 2 with .01 p.c. one secretion slight, the other moderate; 1 with .001 p.c. secretion moderate). In three experiments Ringer's fluid was injected and not adrenaline, and it caused secretion in two.

Ten experiments were made in which Ringer's fluid was injected into .

greater secretion than Ringer's fluid it was injected first. It is possible that in some cases its vaso-constrictor effect may spread to the opposite side of the pad and thus reduce the excitability below the threshold of stimulation by Ringer's fluid.

In February and March we made a number of experiments to determine the effect of conditions (2) and (3), but, as we have mentioned earlier, Ringer's fluid and adrenaline either caused no secretion or caused so little as to be useless for our object.

Reviewing the results of the comparative experiments, it may be doubted whether the method can give completely decisive results, for though it is certain that the excitability of the glands varies in some cats in different parts of the secretory surface, it does not seem possible to determine the degree of difference before injecting the fluids to be compared.

It is of some significance that the secretion caused by $\cdot 001$, $\cdot 01$ and 1 p.c. adrenaline is not very different, but on the whole secretion is rather greater and is commonly of longer duration with $\cdot 001$ p.c. The fact is not easily reconcilable with adrenaline itself causing a secretion. The fact which we think is decisive is that already mentioned in Part I, viz. the restriction of the secretion to the area injected. In none of the experiments referred to above, or to be referred to later, did adrenaline cause secretion outside the injected area. When a small amount of pilocarpine is injected into the mid pad of one foot, its secretory effect spreads to the side pads and then to the toes without causing secretion in any other foot. The depressive action of adrenaline on the response to pilocarpine—the details of which we consider presently—also spreads beyond the injected area. We have seen that $\cdot 001$ p.c. adrenaline usually causes a little more secretion locally than $\cdot 1$ p.c. If adrenaline itself caused secretion it is, we think, inevitable that a $\cdot 1$ p.c. solution would diffuse, or be carried by blood vessels, into the surrounding area sufficiently to make a $\cdot 001$ p.c. solution and so would cause secretion outside the injected area. We have made a few experiments only with solutions stronger than $\cdot 1$ p.c.; in one of these $\cdot 75$ p.c. adrenaline caused secretion and it was strictly local. It follows, we think, that the secretion which adrenaline solution causes in the injected area is due to the fluid in that area, and not to the adrenaline.

The decrease of secretion caused by local injection of adrenaline.

It is clear that adrenaline will tend to reduce secretion of sweat both because it causes great reduction of the blood supply to the glands and

because the decreased blood supply leads to a lower skin temperature. Knauer and Billigheimer⁽²⁾ found in patients suffering from spontaneous sweating that adrenaline stopped the sweating for a considerable time. Billigheimer⁽³⁾ adopting Dieden's view of the presence of inhibitory fibres in the posterior roots attributed the action of adrenaline to its stimulating these nerve fibres. It has been shown in Part I that the posterior roots do not inhibit sweating; so that if adrenaline stimulates inhibitory nerve fibres, they must belong to the sympathetic system. Billigheimer's conclusion was based on the following observations. Into patients he injected pilocarpine on one day, and pilocarpine sometime after adrenaline—on an average 15 to 20 minutes—on another day. He found that the beginning of the secretion was later in the latter case by a time varying from 2 to 20 minutes. He considered that the retarding action was not due to vaso-constriction, since this in some cases had passed off before the pilocarpine was injected. This argument overlooks that though the vaso-constriction had passed off the skin would probably not have recovered the normal temperature. In his experiments 1 mg. of adrenaline was injected sub-cutaneously, so that the amount reaching the sweat glands through the circulation would be very small. Thus on Billigheimer's hypothesis the supposed inhibitory nerve fibres must be very responsive to adrenaline, but when he injected adrenaline after pilocarpine he did not find that it had any retarding action, i.e. by this method there was no evidence of inhibitory action.

The experiments mentioned earlier in this paper show that in most cases adrenaline causes decidedly less secretion than does Ringer's fluid, so that it must lessen the secretory response to the stimulus set up by the fluid in which it is dissolved. In order to determine more accurately the degree of depressive action of different concentrations of adrenaline, experiments were made in the following way—Ringer's fluid, or adrenaline of a given percentage, was injected into the mid pad of one foot, and adrenaline of another percentage into the mid pad of the opposite foot. When the secretion caused by Ringer's fluid (if any) had ceased or was only slight (about half an hour later) 1-2 mgms. of pilocarpine were injected sub-cutaneously in the upper abdominal region and the relative degree of secretion in the two feet, and in the different parts of the same foot noted.

The effect of Ringer's fluid varied considerably, sometimes it had a marked depressive effect. An example of this has been given in Part I, Exp. 4, p. 116. In this the secretion caused by Ringer's fluid though fairly free was brief, i.e. the excitability of the gland was not great.

greater secretion than Ringer's fluid it was injected first. It is possible that in some cases its vaso-constrictor effect may spread to the opposite side of the pad and thus reduce the excitability below the threshold of stimulation by Ringer's fluid.

In February and March we made a number of experiments to determine the effect of conditions (2) and (3), but, as we have mentioned earlier, Ringer's fluid and adrenaline either caused no secretion or caused so little as to be useless for our object.

Reviewing the results of the comparative experiments, it may be doubted whether the method can give completely decisive results, for though it is certain that the excitability of the glands varies in some cats in different parts of the secretory surface, it does not seem possible to determine the degree of difference before injecting the fluids to be compared.

It is of some significance that the secretion caused by $\cdot 001$, $\cdot 01$ and 1 p.c. adrenaline is not very different, but on the whole secretion is rather greater and is commonly of longer duration with $\cdot 001$ p.c. The fact is not easily reconcilable with adrenaline itself causing a secretion. The fact which we think is decisive is that already mentioned in Part I, viz. the restriction of the secretion to the area injected. In none of the experiments referred to above, or to be referred to later, did adrenaline cause secretion outside the injected area. When a small amount of pilocarpine is injected into the mid pad of one foot, its secretory effect spreads to the side pads and then to the toes without causing secretion in any other foot. The depressive action of adrenaline on the response to pilocarpine—the details of which we consider presently—also spreads beyond the injected area. We have seen that $\cdot 001$ p.c. adrenaline usually causes a little more secretion locally than $\cdot 1$ p.c. If adrenaline itself caused secretion it is, we think, inevitable that a $\cdot 1$ p.c. solution would diffuse, or be carried by blood vessels, into the surrounding area sufficiently to make a $\cdot 001$ p.c. solution and so would cause secretion outside the injected area. We have made a few experiments only with solutions stronger than $\cdot 1$ p.c.; in one of these $\cdot 75$ p.c. adrenaline caused secretion and it was strictly local. It follows, we think, that the secretion which adrenaline solution causes in the injected area is due to the fluid in that area, and not to the adrenaline.

The decrease of secretion caused by local injection of adrenaline.

It is clear that adrenaline will tend to reduce secretion of sweat both because it causes great reduction of the blood supply to the glands and

because the decreased blood supply leads to a lower skin temperature. Knauer and Billigheimer⁽²⁾ found in patients suffering from spontaneous sweating that adrenaline stopped the sweating for a considerable time. Billigheimer⁽³⁾ adopting Dieden's view of the presence of inhibitory fibres in the posterior roots attributed the action of adrenaline to its stimulating these nerve fibres. It has been shown in Part I that the posterior roots do not inhibit sweating; so that if adrenaline stimulates inhibitory nerve fibres, they must belong to the sympathetic system. Billigheimer's conclusion was based on the following observations. Into patients he injected pilocarpine on one day, and pilocarpine sometime after adrenaline—on an average 15 to 20 minutes—on another day. He found that the beginning of the secretion was later in the latter case by a time varying from 2 to 20 minutes. He considered that the retarding action was not due to vaso-constriction, since this in some cases had passed off before the pilocarpine was injected. This argument overlooks that though the vaso-constriction had passed off the skin would probably not have recovered the normal temperature. In his experiments 1 mg. of adrenaline was injected sub-cutaneously, so that the amount reaching the sweat glands through the circulation would be very small. Thus on Billigheimer's hypothesis the supposed inhibitory nerve fibres must be very responsive to adrenaline, but when he injected adrenaline after pilocarpine he did not find that it had any retarding action, *i.e.* by this method there was no evidence of inhibitory action.

The experiments mentioned earlier in this paper show that in most cases adrenaline causes decidedly less secretion than does Ringer's fluid, so that it must lessen the secretory response to the stimulus set up by the fluid in which it is dissolved. In order to determine more accurately the degree of depressive action of different concentrations of adrenaline, experiments were made in the following way—Ringer's fluid, or adrenaline of a given percentage, was injected into the mid pad of one foot, and adrenaline of another percentage into the mid pad of the opposite foot. When the secretion caused by Ringer's fluid (if any) had ceased or was only slight (about half an hour later) 1-2 mgms. of pilocarpine were injected sub-cutaneously in the upper abdominal region and the relative degree of secretion in the two feet, and in the different parts of the same foot noted.

The effect of Ringer's fluid varied considerably, sometimes it had a marked depressive effect. An example of this has been given in Part I, Exp. 4, p. 116. In this the secretion caused by Ringer's fluid though fairly free was brief, *i.e.* the excitability of the gland was not

none later. If .1 c.c. of pilocarpine .1 to 1 p.c. is injected into the mid pad itself secretion is sometimes obtained although it is not obtained by injecting 1-2 mgms. sub-cutaneously elsewhere, *i.e.* the strength of the stimulus is a factor in the occurrence or non-occurrence of a secretion. The injection of pilocarpine must, however, not be delayed too long, for at the end of an experiment lasting $1\frac{1}{2}$ -2 hours, it is common to find that local injection of even 1 p.c. pilocarpine causes no secretion in the adrenaline area and but a slight secretion elsewhere.

When 1-2 mgms. of pilocarpine are injected first in the abdominal region, and the secretion is fairly free, the effect of injecting adrenaline .01-.1 p.c. into the mid pad is to stop the secretion in it gradually but permanently. Adrenaline .001 p.c. may also stop the secretion, but if it does it usually begins again in 20-30 mins. When the secretion caused by pilocarpine has become slow, the secretion may be stopped in a few seconds by injecting adrenaline. The depressive effect of the injected solutions can also be shown by stimulating the posterior tibial nerve or the lumbar sympathetic, but if gland excitability is high, the stimulus should be weak.

Some experiments were made in order to determine whether the depressive effect of adrenaline was greater as its action was more prolonged. In these 1 to 2 mgms. of pilocarpine were injected sub-cutaneously in the body region 5 mins. after .1 p.c. adrenaline had been injected into the mid pad of one foot, and 25-30 mins. after it had been injected into the mid pad of the opposite foot. It was found that when a secretion was obtained on the mid pad, either there was rather more in that on which adrenaline had acted for the shorter time, or that there was a trifling secretion in this and none in the other pad. In one experiment a .75 solution of "crystallised adrenaline" was used, and pilocarpine 1 p.c. was injected locally into the pads. Injected 9 mins. after the adrenaline it caused a slow but fairly good secretion in the mid pad—which died out in about 15 mins.—a slight secretion in the side pads, and a free secretion in the toes. Injected 20 mins. after the adrenaline into the other mid pad, it caused a slight secretion only. This was the maximum difference obtained; in the other experiments the difference though distinct was slight. There is then a slight decrease of excitability during the action of adrenaline.

On the theory that the depressive action of adrenaline is caused by vaso-constriction the facts given above would be accounted for as follows: Adrenaline causes strong contraction of the arteries in the injected region and thus greatly reduces the blood supply. The production of a secretion

by pilocarpine after adrenaline depends upon the excitability of the glands, the concentration of the pilocarpine and on the degree to which the blood flow around the glands is reduced. Thus 1-2 mgms. of pilocarpine injected sub-cutaneously elsewhere than in the foot will cause secretion if the excitability is above a certain level, but not if it is below this level. Secretion in some cases can still be produced by injecting .1 c.c. of 1 p.o. pilocarpine locally because the stimulus is stronger. Adrenaline .001 p.c. causes less arterial contraction than .01 or .1 p.c. and the contraction is less prolonged. Thus a given amount of pilocarpine causes more secretion after .001 p.c. adrenaline than after .01 or .1 p.c. and as the contraction lasts a shorter time, the secretion which has been stopped by .001 p.c. may begin again. The arterial contraction produced by .01 p.c. adrenaline is approximately maximal, so that .1 p.c. can cause little more, and the depressive action of the two is nearly the same in the injected area, the diffusion in the surrounding area, in sufficient concentration to cause strong contraction, will however be more extensive with .1 p.c. than with .01, so that its depressive effect on secretion will be more extensive. The common absence of effect of 1 p.c. pilocarpine when injected $1\frac{1}{2}$ -2 hours after .1 p.c. adrenaline is due to the protracted vaso-constriction and consequent progressive decrease of excitability.

This explanation makes some assumptions as to the extent and duration of the reduction in blood supply. Before passing to the observations made to test these assumptions we may mention briefly the result of some experiments on the degree of depressive action of other solutions. Barium chloride 1 p.c., though it has usually about as much secretory effect as Ringer's fluid, has after a time a marked depressive effect on excitability. When 1 to 2 mgms. pilocarpine are injected sub-cutaneously elsewhere than in the foot about half an hour after local injection of 1 p.c. barium chloride, it does not as a rule cause any secretion in the injected area. The depressive effect, unlike that of .1 p.c. adrenaline rarely extends to any obvious degree, beyond the injected area; in two cases only the two mid toes secreted less than the others and than the side pads. It may be presumed that 1 p.c. barium chloride has a gradual depressive action on the gland cells. Calcium chloride 1 p.o. in the two experiments tried had a similar depressive action. Pituitrin (Allen and Hanbury's preparation) was injected locally in the strength in which it is sent out. Five experiments were made, in none had it any appreciable depressive action, the secretion indeed in three of the experiments was rather freer in the injected area than elsewhere. One experiment only was made with

maining obvious. It may be noted that this effect of adrenaline is much greater than can be obtained by stimulation of the cervical sympathetic.

The results just described are in general features like those deduced above as occurring in the deeper tissue of the cat's foot. Adrenaline $\cdot 001$ p.c. caused less complete, less protracted and less extensive contraction than $\cdot 1$ p.c.; the latter caused complete contraction of the small veins, and there was little difference in the effect of $\cdot 01$ and $\cdot 1$ p.c. adrenaline except in extent in the area affected. But in most cases the effect on the vessels appeared to be greater than on those of the cat's foot, for in the foot pilocarpine obviously sometimes makes its way to the glands after local injection of $\cdot 1$ p.c. adrenaline since injected elsewhere it may cause secretion; $\cdot 01$ p.c. adrenaline does not cause venous congestion, and pituitrin causes but slight pallor. Possibly pituitrin has a slight secretory effect. Adrenaline $\cdot 1$ p.c. injected into the gum caused pallor lasting more than two hours; here, as in the sub-cutaneous tissue of the cat, there was no cyanosis.

Effect on secretion of clamping the blood vessels. It is known that by stimulating the sympathetic nerves, secretion of sweat can be obtained after death. Restriction of circulation by adrenaline need not then prevent the fluid in which it is dissolved from causing secretion, and we have seen that adrenaline solution may cause secretion lasting 15 to 20 minutes. In order to determine more exactly the effect of restriction and cessation of circulation upon sweat secretion, we made experiments in which the femoral or common iliac or brachial artery was clamped before or after injecting pilocarpine. One to two mgms. of pilocarpine were injected sub-cutaneously into the abdominal region or $\cdot 1$ to $1\cdot 5$ c.c. of $\cdot 01$ to 1 p.c. pilocarpine were injected sub-cutaneously into the mid pad. Here as in other experiments the results varied more or less obviously with the excitability of the glands.

Clamping the common iliac vein had but a small effect on secretory activity during half-an-hour, the longest time we have tried. The secretion became somewhat but not greatly less than on the opposite side. Clamping the femoral artery centrally of the popliteal branch had a considerably greater effect, but it varied with the condition of the glands. Three experiments were made; in two of them, in which the artery was clamped early in the experiment, secretion continued for the half-an-hour during which the clamp was kept on, but it became slow towards the end of the time. In one of these experiments the feet were unpigmented, the foot on the side with artery clamped remained slightly pink, and on pressing it, the pink tinge slowly returned, so that passage of blood to

the skin was not completely stopped. That blood can slowly pass to the foot after clamping the artery was shown by injecting pilocarpine in the body region (cp. Exp. 2).

Exp. 2. Femoral artery clamped. Ten minutes later 2 mgms. of pilocarpine were injected sub-cutaneously in the abdominal region. A trace of secretion appeared on the pad of the foot ten minutes after the injection. The drops steadily increased in size in the course of the next ten minutes, and formed on the toes.

Since clamping the femoral artery stops all direct supply of blood, it is clear that secretion can go on for a considerable time with a slow circulation and a small oxygen supply. In the third experiment the artery was clamped after other observations; in this case (cp. Exp. 5) the secretion was stopped at once except for a mere trace. The greater effect here was no doubt partly due to lowered excitability and less strong stimulus, but probably the degree to which the blood supply is cut off varies in different cats and varies with the blood pressure.

On clamping the common iliac artery we did not find that pilocarpine injected in the body region caused secretion in the foot in the $8\frac{1}{2}$ mins. before unclamping (cp. Exp. 3). The effect produced by clamping the artery, when pilocarpine has been given before clamping, depends mainly upon the excitability of the glands. Exp. 3 shows the result when the excitability is fairly high.

Exp. 3. The left common iliac artery was clamped. Two minutes later 1.5 mgms. of pilocarpine were injected sub-cutaneously in the body region. The artery was unclamped after $8\frac{1}{2}$ mins. during which time there was no secretion; the secretion on unclamping rapidly became copious in all parts of the foot. The foot was wiped, and the artery clamped.

Time after clamping	State of foot
3 mins.	Good secretion on whole of pad, none in toes
5 "	Very slight secretion on pad
8 "	No secretion

On unclamping the artery the secretion in 3 mins. became copious on the pad and slight on the toes. We may note that unless there has been a previous injection into the pad (as in Exp. 5) clamping the artery has more effect in decreasing the secretion in the toes than in the pad.

It will be seen that notwithstanding cessation of the circulation, the secretion went on freely for about three minutes, then rapidly diminished and stopped in about eight minutes. A higher degree of excitability or a larger amount of blood in the limb at the moment of clamping the artery would no doubt allow secretion to go on longer than in this experiment.

On repetition of the clamping, the time required to stop the secretion usually becomes less and less, until it is stopped at once (cp. Exp. 4), and if excitability is low at the beginning of an experiment, clamping may stop it at once.

Exp. 4. Observations had been made on the effect of local injection on the fore foot. The left iliac artery had been elamped for a time sufficient to cause congestion of the foot and a copious secretion produced by injecting .1 c.c. 1 p.c. pilocarpine into the mid pad. The following observations were then made:

0	mins.	Artery elamped; foot remained red.
5	"	Secretion stopped.
6	"	Common iliac vein elamped.
7	"	No secretion. Artery unclamped. In 3 mins. copious secretion.
12	"	Artery elamped, vein unclamped. Press the foot, the pallor produced slowly (30 secs.) gives way to a pink tint.
14	"	Secretion copious.
14½	"	" moderate, wipe the foot.
15½	"	" very slight.
16	"	No secretion; foot is a blue red.
20	"	No secretion. Unclamped artery; secretion copious in 3 to 4 mins.
35	"	Secretion moderate to good. Clamp artery and wipe foot. No secretion in 6 mins.
41	"	Unclamp artery. Fairly good secretion in 2 mins.
45	"	Clamp artery. Trace of secretion in ½ min. which rapidly disappears.
48	"	Unclamp artery. Moderate secretion in 4 mins.

From these and similar experiments it follows that both *adrenaline* and clamping the artery stop secretion in a time depending upon the excitability of the glands. When *pilocarpine* is injected into the pad of the foot a short time after clamping the artery, and whilst the artery remains clamped, the occurrence or non-occurrence of a secretion depends similarly on the gland excitability. If it is fairly high, free secretion goes on for a few minutes, it then diminishes, at first rapidly and then slowly. If excitability is low, there is either no secretion, or there is none for several minutes and then a very slow secretion commences. The explanation of this retarded secretion we shall deal with presently, we may first give an example of an experiment which shows it and which shows the relative effect of restriction of blood supply by *adrenaline* and by clamping the common iliac artery.

Exp. 5. Ringer's fluid caused no secretion in the mid pads. The common iliac artery was clamped on one side and .1 c.c. .1 p.c. *adrenaline* injected into the mid pad of the opposite side. A few minutes later .1 c.c. of .1 p.c. *pilocarpine* was injected into the mid pad of each foot.

Mins. from pilocarpine injection	Artery elamped. Secretion	Adrenaline in mid pad. Secretion
3	None	None
10	None	Slight on part of mid pad
20	Trace on whole of pad, moderate on toes but unequal	None on mid pad, slight on side pads, good on toes
29	Secretion less	Moderate on toes
30	(Unclamp iliac artery)	
31½	Good	
38½	(Clamp femoral artery)	
42	None	
48½	Trace	Slight on 2nd and 3rd toes
50	(Unclamp femoral), good in a few minutes	

It will be seen that in this experiment, the slight secretion began rather earlier in the mid pad which had been injected with adrenaline than in that in which the blood supply had been stopped by arterial clamping. Although on account of possible variation in excitability no great stress can be laid on the earlier occurrence of secretion after adrenaline, it may fairly be inferred that the effect of adrenaline and of decrease of blood supply is so nearly the same as to leave no room for appreciable inhibitory effect of adrenaline. On the adrenaline side the local vaso-constriction continued and gradually involved the rest of the foot so that the secretion gradually decreased.

There are some minor effects of restriction of blood supply which are worth notice. These chiefly relate to its after effects. When a secretion is going on it sometimes happens that the after-effect of clamping the common iliac artery is to produce a rather freer secretion than on the side the artery of which has not been clamped, and a rather freer secretion than occurred before clamping. So far as we have seen this is only marked when the excitability is low. Exp. 6 is an example.

Exp. 6. Ringer's fluid injected into the mid pads caused no secretion, 1.5 mgms. of pilocarpine injected under the body skin caused a moderate secretion, after this had gone on for 25 mins. the feet were wiped and the left common iliac artery clamped for 6 mins.; during this time there was no secretion on the clamped side. On unclamping the artery, the secretion was rather more than on the opposite side and rather more than it was before clamping. After repeating the clamping and unclamping, .1 c.c. of .2 p.c. pilocarpine was injected into each mid pad; the secretion was slow and moderate. The artery was clamped for 15 mins.; a trifling secretion went on for a minute or two only; the foot was pale. On unclamping, the foot became intensely red and the secretion was distinctly greater than on the opposite side and than it was before.

The greater secretion on the side on which the artery had been clamped than on that on which it had not, is partly due to the absence of fatigue in the former; in the latter, fatigue is caused by the continued secretion. But the increase of secretion over that before clamping can only be due to the increased blood flow which follows anæmia. The increase does not always occur; this lack of constancy is partly due to the fact that the degree of hyperæmia caused by previous clamping of the common iliac artery for a given time varies in different cats and varies with the blood-pressure. There is another factor which we think influences the result, viz. a slight decrease of excitability during anæmia. Three experiments were made on this point in the following way. The common iliac artery was clamped on one side for 5 mins. and on the other for 30 mins. Two minutes before unclamping the arteries, 2 mgms. of pilocarpine were injected sub-cutaneously in the body region. The

Exp. 4. Observations had been made on the effect of local injection on the fore foot. The left iliac artery had been clamped for a time sufficient to cause congestion of the foot and a copious secretion produced by injecting .1 c.c. 1 p.c. pilocarpine into the mid pad. The following observations were then made:

0	mins.	Artery clamped; foot remained red.
5	"	Secretion stopped.
6	"	Common iliac vein clamped.
7	"	No secretion. Artery unclamped. In 3 mins. copious secretion.
12	"	Artery clamped, vein unclamped. Press the foot, the pallor produced slowly (30 secs.) gives way to a pink tint.
14	"	Secretion copious.
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From these and similar experiments it follows that both adrenaline and clamping the artery stop secretion in a time depending upon the excitability of the glands. When pilocarpine is injected into the pad of the foot a short time after clamping the artery, and whilst the artery remains clamped, the occurrence or non-occurrence of a secretion depends similarly on the gland excitability. If it is fairly high, free secretion goes on for a few minutes, it then diminishes, at first rapidly and then slowly. If excitability is low, there is either no secretion, or there is none for several minutes and then a very slow secretion commences. The explanation of this retarded secretion we shall deal with presently, we may first give an example of an experiment which shows it and which shows the relative effect of restriction of blood supply by adrenaline and by clamping the common iliac artery.

Exp. 5. Ringer's fluid caused no secretion in the mid pads. The common iliac artery was clamped on one side and .1 c.c. .1 p.c. adrenaline injected into the mid pad of the opposite side. A few minutes later .1 c.c. of .1 p.c. pilocarpine was injected into the mid pad of each foot.

Mins. from pilocarpine injection	Artery clamped. Secretion	Adrenaline in mid pad. Secretion
3	None	None
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29	Secretion less	Moderate on toes
30	(Unclamp iliac artery)	
31½	Good	
38½	(Clamp femoral artery)	
42	None	
48½	Trace	Slight on 2nd and 3rd toes
50	(Unclamp femoral), good in a few minutes	

small blood and oxygen supply, but some supply of oxygen is necessary for continued secretory activity.

On unclamping the artery of a hind limb, there is, as is known, flushing of the skin more or less in proportion to the duration of the anæmia, the increased blood flow tends to cause a freer secretion than occurred before the clamping. This may be counterbalanced by the slight progressive decrease of excitability occurring during anæmia. Up to the limit of duration of anæmia tried—about half an hour—the excitability is rapidly recovered on re-establishment of the circulation.

A drop of adrenaline ·1 p.c. locally injected into the ear of the rabbit causes local contraction of the veins. When injected in rather greater amount it causes extensive venous contraction and stagnation in part of the capillary area. Adrenaline ·1 p.c. locally injected into the foot causes a bluish flush of the skin, due to contraction of the small veins, as well as of the arteries. The fat tissue in which the glands are imbedded become, however, almost bloodless. Thus the state of the dermal capillaries does not necessarily show the state of the sub-cutaneous capillaries.

Adrenaline causes much greater contraction of the veins in the rabbit's ear than is caused by stimulating the cervical sympathetic nerve.

Pituitrin and some other vaso-constrictor substances when injected into the foot appeared to produce insufficient vaso-constriction to seriously influence secretory activity.

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FORMATION AND DISTRIBUTION OF PHOSPHATES
IN MUSCLE. BY L. B. WINTER, B.A. (*Scholar of Christ's
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EMBDEN and others(1) have given evidence by chemical methods that the setting free of lactic acid in muscle is accompanied by the setting free of an equimolecular amount of phosphoric acid, both acids arising from hexose phosphate. Fletcher and Hopkins(2) had previously shown that in muscle in which survival changes had been arrested the lactic acid is minimal, and that it increased to a definite maximum during rigor. Macallum, using the ammonium molybdate method for the detection of inorganic phosphates in tissues under the microscope, has described phosphates as being present in the dim band (disc Q, anisotropic disc) only. His specimens were presumably of muscles in rigor. From these results it follows that a difference in the phosphoric acid content of the dim band in "rigor muscle," and that in which survival changes have been arrested, should be visible in microscopic specimens. We have made some observations to test how far this is true.

Method for the detection of phosphoric acid. The test which is used to detect inorganic phosphates in tissues is the yellow precipitate of ammonium phospho-molybdate which is formed when ammonium molybdate in the presence of nitric acid is added to a phosphate solution. The yellow tint produced is, however, too faint for satisfactory localisation, and various methods have been used to overcome this difficulty. Lilienfeld and Monti(3) used pyrogallol after the molybdate, which gives rise to a brown or black molybdic oxide. Macallum(4) has pointed out objections to this method and employed instead phenylhydrazine hydrochloride 1-4 p.c. This reacts at once with the phospho-molybdate to give the green-black oxide of molybdenum. We have used a 3 p.c. solution of potassium ferrocyanide which produces a blue compound. When added to phospho-molybdate in a test tube it produces a precipitate which is much more finely divided than that obtained by phenylhydrazine hydrochloride, and it has the advantage of not being acid. When added to ammonium molybdate in absence of acid no change of

colour occurs, but in presence of nitric acid a dark red colour is produced. This makes no difficulty in the preparations for if carefully washed no red colour appears. For our purpose it was necessary to use as low a concentration of nitric acid as practicable in the ammonium molybdate solution, since acid would tend to set free phosphoric acid. We therefore experimented with various solutions to find the least concentration of nitric acid which would show the presence of phosphorus with certainty. Two standard solutions were made up:

A. Ammonium molybdate 13 grams per 100 c.c. dissolved in distilled water by the aid of heat.

B. Concentrated nitric acid to which an equal volume of distilled water was added.

Taking constant amounts of A we added increasing minimal amounts of B until the detection of phosphorus in the dim bands was regularly observed. The solution which we finally adopted was made by taking 10 c.c. of A and adding to it 0.4 c.c. of B. The mixture was well shaken and the precipitate allowed to settle; the clear fluid was then pipetted off and used; it was prepared each day to avoid possible alterations in concentration. To make certain that immersion of the tissue in this concentration of acid would not set free any iron compound which would subsequently react with the potassium ferrocyanide we carried out control experiments. The tissue was immersed for two hours in nitric acid of the same concentration (0.4 c.c. in 10 c.c.) in water: after washing the tissue and transferring it to the potassium ferrocyanide solution no blue precipitate or coloration could be detected. Owing to the use of potassium ferrocyanide it is important to note that no iron must come into contact with the tissues.

Method of preparing the muscle specimens. Our first experiments were made on the sterno-cutaneous muscle of the frog; but owing to its thickness, the exact differentiation of the structure under the microscope was difficult, and it was not easy to remove specimens from the frogs by means of glass needles which we were compelled to use in order to maintain the iron-free technique. We therefore decided to use the leg muscles of insects, and found that the cockroach (*Periplaneta Americana*) was most suitable for the purpose. The muscles of this species are large and easily teased; also the structure is very much more definite than that of frog's muscle.

Our primary object was to compare the phosphate content of normal resting muscle with that of muscle in rigor. Fletcher and Hopkins⁽²⁾ have shown that lactic acid is present in minimal amount in muscles

which have been ground in ice-cold alcohol and we may assume that in the same conditions phosphoric acid is not liberated. We obviously could not grind up the muscle, and alcohol penetrates tissues slowly, so that the method of preventing survival change had to be modified. We placed the fresh insect muscle in ice-cold formol (4 p.c.) which penetrates rapidly, considering that, if this did not prevent all survival change, it would prevent it sufficiently to allow comparison with muscle which had not been similarly treated. The muscle fixed fresh in formol we speak of as "resting" muscle.

In the preparation of "rigor muscle" we thought it better not to employ beat or chloroform since these might destroy the permeability of the muscle structures and cause diffusion of phosphoric acid. In fact we found that after treatment with chloroform water, the blue colour was more or less diffuse throughout the fibres. The method we adopted was to tease out the muscle and leave it in air for an hour. This would allow survival changes to go on, and rigor would probably set in on placing the fibres in the molybdate solution.

We took pairs of limbs, one was placed in ice-cold formol for a quarter of an hour; the muscle then removed, teased out with glass needles in water as rapidly as possible, and placed in the molybdate solution. (Traces of formol do not prevent the precipitation of the phosphomolybdate.) In this it was left for half an hour, then placed in distilled water and thoroughly washed in several changes of water for half an hour. It was then transferred to potassium ferrocyanide, in which a blue colour rapidly developed. After half an hour it was washed well and mounted in glycerine jelly. The muscle of the other limb was teased out, left in air for an hour, then treated with molybdate solution and ferrocyanide of potassium as above.

Results. In the rigor muscle the dim band was of a markedly blue tint. The sarcoplasm, including in this the interstitial substance between the fibrils, was uncoloured. In the region between the dim bands, the accumulation of interstitial substance and the greater thinness of the fibrils makes it difficult to be certain of the total absence of coloration in the fibres, but on examination with 1/12 oil immersion we could see none, and we conclude that, as Macallum stated, the inorganic phosphates are confined to the dim band.

In the resting muscle the dim bands were coloured a faint blue, the rest of the muscle substance being uncoloured. How far this indicates the presence of free inorganic phosphates in the living muscle we cannot say, for some may have been set free in killing the muscle with formol,

and some were probably set free by the nitric acid of the molybdate solution. We found that if the resting muscle was left in the molybdate solution for two hours instead of for half an hour the blue stain was as deep as in the rigor muscle.

Miller and Taylor(6) describe the reduction of ammonium molybdate in acid solution by a variety of organic compounds, resulting in the production of a deep blue colour. We found occasionally when the tissue remained too long in the molybdate solution that the yellow colour took on a greenish tinge. This may have been due to the formation of the above blue compound in small amount, but if the tissue is not left in the molybdate solution for more than two hours or heated above $30^{\circ}\text{C}.$, we find no greenish colour develops. The blue colour of course does not appear until the tissue is placed in the potassium ferrocyanide solution.

It is now generally accepted that in vertebrate muscle free phosphoric acid and lactic acid arise from the same precursor. In view of this it seemed interesting to decide whether lactic acid is also produced in the muscles of the cockroach. For this purpose we employed Hopkins' delicate thiophene reaction. The muscles of four cockroaches were taken, left at $40^{\circ}\text{C}.$ for half an hour, extracted with alcohol and treated with charcoal. Clear evidence of the presence of lactic acid was obtained. We tested the muscles of these insects for potassium, using Macallum's method(5) and found that it was localised as he described, *i.e.* in the dim bands.

We made also some observations on the unstriated muscle of the frog's bladder. The bladder was treated in the manner given above for the rigor preparation of striated muscle, except that it was not teased and that it was left in the acid molybdate solution for two hours. The muscle plexus showed a faint blue tint only, and this was evenly distributed throughout the muscle fibres. We may assume that if a precursor of phosphoric acid had been present, it would have been split up by this treatment. In any case, it seems clear that unstriated, unlike striated, muscle sets free little (if any) phosphoric acid in dying.

Remarks. The quick contraction of striated muscle has been attributed by many authors to setting free of lactic acid. Embden considers that the precursor of lactic acid contains also phosphoric acid. The capacity for quick contraction is universally attributed to the existence of the dim bands. The facts we have given above, *viz.*, that phosphoric acid is set free in the dim bands in rigor, and that no appreciable amount is set free in unstriated muscle on dying are, so far as they go, in favour of Embden's theory and suggest that phosphoric acid must play some important part in the production of quick contraction.

SUMMARY.

1. Some modifications are described of the method for localising phosphorus in tissues.

2. By the aid of these it is shown that the amount of uncombined phosphate in striated muscle is increased in rigor. An increase was not found in unstriated muscle.

We wish to thank Professors J. N. Langley and F. G. Hopkins for kindly criticism and help in preparing this paper for publication.

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THE RELATION OF NERVE-SUPPLY AND BLOOD FLOW TO SWEATING PRODUCED BY PILOCARPINE.

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LANGLEY(1) has recently published a paper confirming an observation originally made by himself and Anderson(2) that the action of pilocarpine on the sweat glands of the cat's foot was not dependent on the integrity of the nerve supply. Langley finds that it is always possible to evoke sweat secretion by the injection of pilocarpine directly into the pad of the foot long after the sciatic nerve has degenerated, but he considers that nerve degeneration is not without its effect, as it leads to a condition of diminished excitability, in which the sweating on the denervated paw is conspicuously less than on the normal paw. The view that pilocarpine acts directly on the gland cell is not accepted by all. Lewis(3) observed that in certain patients the production of sweat by pilocarpine was abnormally great, and he concluded that this indicated a hyperexcitability of the peripheral terminations of the sweat nerves. Rowntree(4) states that the effect of pilocarpine on the sweat glands is lost after section of the spinal nerves. The experimental evidence for this view is fully reviewed by Langley. My experiments, which began in December, 1920, soon confirmed the observation of Langley and Anderson that pilocarpine may cause sweating in the complete absence of innervation; they showed, however, that denervation affected the pilocarpine response, and that while degeneration of the mixed nerve supply led to diminution of the sweat secretion, degeneration limited to the sympathetic fibres increased it.

Methods. The production of sweat on the pad of the cat's foot is not a phenomenon which readily lends itself to quantitative observation. It is possible to weigh the sweat produced during a given time by collecting it on small weighed pieces of dry filter paper. A few experiments have been performed in this way; the procedure, however, is laborious, and subject to so large an experimental error, that it cannot be used to detect differences not immediately obvious to the naked eye. In healthy kittens from 2-3 months old, the pads of the feet are nearly

always soft, and sweat readily when pilocarpine is given subcutaneously; so far as can be seen by careful inspection the amount produced on one fore or hind paw is identical with that on the corresponding paw of the other side. Kittens of this age have been used exclusively, and the effect of denervation on the sweat secretion of one paw has been judged by comparing it with the corresponding normal paw under conditions as uniform as possible. Older cats usually have horny pads, which often sweat very feebly in response to pilocarpine and sometimes unequally. The thickness of the pads does not appear to be associated entirely with age, for one kitten which only ran about on a smooth floor, and was kept in a cage for the greater part of the day, retained its power to sweat profusely when almost fully grown. In all except the earlier experiments the dose of pilocarpine used was regularly 0.5 mg. of the nitrate, and this was injected subcutaneously into the loose skin of the flank. Such a dose causes a kitten no distress, and if patiently handled the animal offers no resistance to the examination of its paws. The routine procedure was to inject this dose and to leave the kitten until salivation began about five minutes later, when the paw under investigation was closely watched for 8-10 minutes in comparison with the corresponding normal paw. The time of the first appearance and the total amount of sweat were carefully noted.

Sweating described as "profuse" represents an amount (collected from one paw) in the neighbourhood of 6-9 mgm.; "marked sweating" about 3-6 mgm., and "slight sweat" about 1-3 mgm. It should be understood, however, that my conclusions in no case depend on the observation of slight differences in the secretion of the two paws. Differences recorded as significant were such as to be immediately obvious to any observer. All operative procedures were carried out under deep anaesthesia with ether and with full aseptic precautions.

Sweating after nerve degeneration.

Section of the sciatic nerve. The effect of section of the sciatic nerve has been fully investigated in nine kittens, and the response to pilocarpine of the denervated paw appears to pass through some or all of three stages. As a rule the secretion during the first few days following the operation appears more rapidly and is much more abundant than that on the normal hind paw. After this stage of exaggerated secretion there is a second stage of variable secretion, in which there is no regularity in the difference between the two paws; whereas on one day the denervated paw may sweat only slightly, a few days later it again sweats more

copiously than the normal paw. Finally, often after four or six weeks, there is a third stage of diminished secretion, in which the denervated paw remains dry in response to the injection of 0.5 mgm. pilocarpine, and only sweats slightly in response to larger doses, which produce a maximal effect on the normal paw. Table I gives an example of the three stages. Six observations are chosen out of the 17 which were made.

TABLE I. Kitten 1. Right sciatic nerve cut.

	Days after operation	Response of right hind paw when 0.5 mgm. pilocarpine injected subcutaneously
Stage I	2	Sweats profusely
	8	
Stage II	18	Remains dry while normal sweats Sweats well while normal is dry
	26	
Stage III	42	Remains dry while normal sweats Remains dry while normal sweats well
	47	

The duration of these different stages varies greatly in different kittens. In two kittens the condition of diminished secretion was present 48 hours after the operation; in a third the condition of exaggerated response, which normally disappears in a week or two, was prolonged until the kitten was killed 10½ weeks later. The different durations of these stages for the nine kittens are set out in Table II; the times given are approximate, as observations were not made with sufficient frequency for close accuracy.

TABLE II. Section of right sciatic nerve.

(Times stated are calculated from the day of operation.)

Exp.	Period of increased secretion				Period of variable secretion	Period of decreased secretion
1	2 weeks	3rd-6th week	6th week onwards
2	1 week	2nd-6th "	6th "
3	1 "	2nd-3rd "	4th "
4	None	None	Present after 48 hours
5	1 week	"	2nd week onwards
6	To end of exp. 11 weeks				"	None
7	1 week	2nd-7th weeks	(Not observed)
8	None	1st-3rd "	"
9	"	None	Present after 48 hours

Section of the brachial plexus. Four experiments were carried out in which the nerves to the fore limb were divided, and in the case of two of the kittens, the phases of exaggerated, variable, and diminished sweating were all seen, just as after division of the sciatic. In the other two, only the stage of diminished secretion was observed.

In the large majority of cases, then, section of the sciatic nerve or of the brachial plexus is followed, sooner or later, by decreased response to pilocarpine of the corresponding sweat glands. On this point my

observations confirm Luchsinger(5) and Langley(1). The facts, however, that, in exceptional cases, the opposite condition may prevail, in which section of the sciatic is followed by a permanently increased response, and that in many cases there is a delay of many weeks in the appearance of the decreased response, make it clear that reduced excitability is not an immediate or inevitable consequence of the disappearance of the secretory nerve endings.

Extirpation of the stellate ganglion. In the case of the fore limb of the cat, Langley has shown that the postganglionic fibres running to the sweat glands all originate from cells in the stellate ganglion. The extirpation of the ganglion has been carried out in five kittens, Anderson's method(6) being followed. In each case the completeness of the extirpation was verified by careful dissection when the animal was killed at the conclusion of the experiments. The effect on the pilocarpine response was quite unlike the effect of complete denervation. No succession of stages was observed in an effect which remained practically constant during the whole period for which observations were continued. In three kittens the sweat seen on the operated paw after an injection of pilocarpine greatly exceeded that produced in the normal fore paw. In a fourth the sweating did not differ appreciably in the operated and normal paws, and in a fifth there was equality in the majority of the observations. In the case of this last kitten only, a few experiments showed appreciably greater sweating on the normal paw than on the operated paw. Table III summarises the observations made.

TABLE III. Extirpation of the stellate ganglion of one side.
Response to 0.5 mgm. pilocarpine of operated paw in contrast with normal.

Exp.	Duration of observations	Number of observations	No. of times response was		
			Exaggerated	Unchanged	Diminished
10	20 weeks	23	19	4	—
3	21 days	6	2	4	—
7	48 "	10	7	2	1
8	40 "	7	6	1	—
5	77 "	12	—	8	4

This contrast between the effect of extirpating the stellate ganglion and that of dividing the whole mixed nerve supply was confirmed in two of the kittens, from which one stellate ganglion had been extirpated, by subsequent division of the brachial plexus of the opposite side. The same differences in the response to pilocarpine were seen in both animals; profuse sweating was observed on the side deprived of the stellate ganglion, while the opposite paw, completely denervated, showed

the same size, of the pattern described by Dale and Richards(8), and these were connected to small bellows recorders of equal sensitiveness.

Several records were then taken of the volume changes of the two limbs in response to 0.01 mgm. histamine and to 0.001 mgm. acetylcholine. In some cases the plethysmograph responses were obtained without recording the blood pressure. No operative procedure being involved, the experiment could be repeated at intervals of days or weeks. For such experiments ether was administered by an open mask, and the injections were made with a hypodermic needle into an ear vein¹. The results with two kittens were as follows:

A. Exp. 10.

(a) Fore paws. Right stellate ganglion extirpated 20 weeks previously. Left brachial plexus cut three weeks previously. Pilocarpine response of the fore paws given in Table IV.

Plethysmograph response (see Fig. 1).

Injection	Right fore limb	Left fore limb
0.01 mgm. histamine	Dilatation	Constriction
0.001 mgm. acetylcholine	Dilatation	Dilatation

(b) Hind paws. Right sciatic nerve divided. Seventeen observations on pilocarpine response during 10½ weeks; in 16 cases the sweating of the denervated paw was much greater than that of the normal paw. In the plethysmograph an injection of histamine caused a slight dilatation of the normal limb and an unusually large dilatation of the operated limb.

B. Exp. 11. Left brachial plexus cut.

(a) Observations during early days following section. Three observations on pilocarpine response during eight days following the operation. In each case sweating was exaggerated on the operated side.

Plethysmograph response, 8th day.

Injection	Normal leg	Operated leg
Histamine	No change	Large dilatation
Acetylcholine	Dilatation	Dilatation slightly greater

(b) Observations late after section. On the 37th day after the operation, the normal paw sweated well in response to pilocarpine, the paw of the operated side not at all.

Plethysmograph response, 37th day.

Injection	Normal leg	Operated leg
Histamine	Slight dilatation	No appreciable change
Acetylcholine	Dilatation	Dilatation slightly greater

Looking at observation (a) of the first experiment, we see (Fig. 1) that in response to an injection of histamine the right leg expanded and the left leg constricted. Following the interpretation of Dale and Richards(8) it must be supposed that the capillaries of the right leg were in a state of tone, so that the injection of histamine caused an expansion of the limb due to a relaxation of that tone. On the other hand

¹ These experiments were kindly performed by Dr H. H. Dale.

in the left leg the capillaries were so completely lacking in tone that the only effect on the limb volume was a passive shrinkage due to the coincident fall in general blood pressure. Reference to Table IV shows that the leg in which the capillary tone was marked was that in which the response of the sweat glands to pilocarpine was profuse, while in the other leg, in which capillary tone was lacking, the sweat glands responded only slightly or not at all. There was no such correspondence in this kitten, or in any other, between the sweating evoked by pilocarpine and the dilator response in the plethysmograph to acetyl-choline.

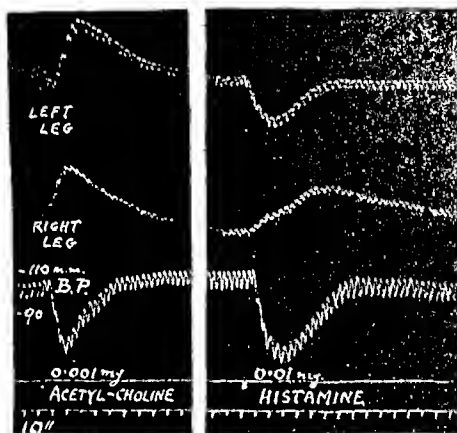


Fig. 1. Kitten. Rt stellate ganglion extirpated 20 weeks previously Left brachial plexus cut 3 weeks previously. The two upper tracings show the volume changes in the fore legs. See text.

This substance has been shown by the work of Reid Hunt (9), Dale and Richards (8) and Krogh (10) to exert its dilator effect mainly on the arteries. The results of a series of observations are summarised in Table V. The vaso-dilator effect of histamine in each partially or completely denervated limb was observed under ether during a period in which the sweating response to pilocarpine was constant.

The experiments described in the table show that in three kittens, 11, 12, and 3, coincidently with the change in the sweating response to pilocarpine after section of the mixed nerve supply, a change in the

TABLE V.

Pilocarpine response + = profuse sweating; as great as or greater than normal.
 " " - = greatly diminished or suppressed sweating.
 Histamine response + = large vaso-dilatation, greater than that of normal paw.
 " " - = vaso-dilatation very small, or absent.

Exp.	Limb	Operation	Time elapsed since operation	Response to pilocarpine	Response to histamine
10	Right fore	Stell. ganglion extirpated	20 weeks	+	+
	Left fore	Brach. plexus cut	3 "	-	-
	Right hind	Sciatic nerve cut	10½ "	+	+
7	Right fore	Stell. ganglion extirpated	6 "	+	+
	Left fore	Brach. plexus cut	1 week	-	-
5	Right fore	Stell. ganglion extirpated	11 weeks	+	+
	Right hind	Sciatic nerve cut	10 "	-	-
11	Left fore	Brach. plexus cut	1 week	+	+
	"	" "	5 weeks	-	-
12	"	" "	2 days	+	+
	"	" "	40 "	-	-
3	Right hind	Sciatic nerve cut	4 "	+	+
	"	" "	7 weeks	-	-
2	"	" "	4 days	+	+
1	"	" "	6 "	+	+

histamine response was observed. During the first week following the operation, the denervated limb in each case showed both exaggerated sweating to pilocarpine and an exaggerated vaso-dilatation with histamine. After an interval varying from four to six weeks, the sweating response was imperceptible, and the vaso-dilatation to histamine had disappeared; the vaso-dilatation produced by acetyl-choline, however, was still the same as during the first week, the dilatation in the operated limb being on both occasions greater than in the normal limb. Similarly, the undiminished or exaggerated sweating after extirpation of the stellate ganglion is shown to be accompanied by a large vaso-dilatation of the limb to histamine, which was present in kittens 10, 7, and 5, 20 weeks, 6 weeks and 11 weeks respectively after the extirpation. The correspondence which the table records between the two phenomena for each operated limb was not always seen in a normal limb. Often a normal limb, which sweated well with 0.5 mgm. pilocarpine, showed little or no vaso-dilatation with histamine; but in such a limb the peripheral vaso-dilator action is probably complicated by secondary effects due to reflex stimulation of the vaso-motor centres, and to excitation of these centres by the anæsthetic.

Factors in the pilocarpine response.

Trophic changes in the skin. After degeneration of the mixed nerve supply, when the sweating produced by pilocarpine was diminished or

absent, the pads took on a dry and scaly appearance, which was not previously seen. In addition, the effect of unusual friction on the skin of the limb was much greater when the sweating response was small than at other times. After division of the brachial plexus of one side, the animal walks, not on the pad, but on the extensor surface of the wrist; and in two kittens in which this operation was carried out, the condition of the skin of this surface varied almost simultaneously with changes in the sweating to pilocarpine. The kittens were from the same litter, and the operations were performed on the same day. They ran about together on the smooth floor of the same room for the next few weeks. After $2\frac{1}{2}$ weeks, in one kitten the sweating to pilocarpine was still large in amount, and the skin on the extensor surface which came in contact with the floor retained its covering of hair and was completely elastic. At the same time, in the other kitten the denervated limb failed to sweat with pilocarpine, and within a day or two the skin of the extensor surface lost its covering of hair, and became inelastic and indurated. Less than a week later the sweating response reappeared, and the induration of the skin for the most part passed off.

Anæsthetics. Adrenaline. It has been shown that the effect of section of the whole nerve supply in diminishing the pilocarpine response is often greatly delayed, and that a period is seen in which the secretion on the denervated paw may change from a diminished response on one day to an augmented response some days later. On studying the records of different kittens several instances were found in which, after ether had been administered for a period of about an hour without operation, the sweating in a completely denervated paw, on the next day and for the few days following, was profuse. Six experiments were carried out with the direct object of testing this observation, and it was found that, provided the response to 0.5 mgm. pilocarpine was not entirely absent in the fully denervated paw, the induction of ether anæsthesia led to an augmented response 24 hours after the anæsthetic was given. Observations on one kitten are recorded in Table VI.

Of the six experiments four were made on one kitten, and the augmented response was observed in three of these; a fifth, made on a second kitten, showed the same effect; but in a sixth, on a third kitten, it was not perceptible. The negative results were seen in those cases in which, during the few days previous to that on which the anæsthetic was given, the response to 0.5 mgm. pilocarpine was entirely absent and the paw looked scaly and unusually dry. In considering the explanation of this phenomenon, the most likely clue seemed to be afforded by Elliott's

TABLE VI. Kitten, Exp. 2. Right sciatic nerve divided.

Day after sciatic section	Sweating of denervated paw with 0.5 mgm. pilocarpine subcutaneously
1, 2, 4, 6	Profuse and definitely greater than normal
8	Slight, but equal to normal
11	Less than normal
12 (ether anaesthesia 1 hour)	
13	Profuse, much greater than normal
18, 19	Slight, greater than normal
25	Less than normal
26	None
28	Less than normal
(Ether anaesthesia 1 hour)	
29	Profuse, much greater than normal
42	Absent

observation (11) that anaesthesia with ether leads to suprarenal exhaustion. It was conceivable that an increase in the circulating adrenaline, following the administration of ether, was responsible for the augmented pilocarpine response. An attempt was, therefore, made to obtain a similar augmentation by the subcutaneous administration of adrenaline. The results with two kittens were as follows:

A. Exp. 2. Right sciatic nerve cut 44 days previously.

	Subcutaneous injection	Sweating on	
		R. hind paw	L. hind paw
3 days later	0.5 mgm. pilocarpine	Nil	Nil
4	"	"	Trace
5	1.0 mgm. " adrenaline	"	"
6	"	"	"
7	0.5 mgm. " pilocarpine	Marked	Marked
14	"	"	Nil

B. Exp. 1. Right sciatic nerve cut 44 days previously.

3	0.5 mgm. pilocarpine	Trace	Sweating
4	"	Nil	"
4	0.8 mgm. " adrenaline	"	"
	0.5 mgm. pilocarpine (4½ hrs. later)	Nil	Marked
5	1.0 mgm. " adrenaline	"	"
	0.5 mgm. pilocarpine (5 hrs. later)	Sweating	Marked
6	1.0 mgm. " adrenaline	"	"
	0.5 mgm. pilocarpine (5 hrs. later)	Marked	Marked

In both cases the subcutaneous injection of 1 mgm. adrenaline daily for three days evidently increased appreciably the response of the denervated sweat glands to pilocarpine. In Exp. A, it will be noticed that the effect of adrenaline was produced in both paws, normal as well as denervated, and that this restorative effect was still pronounced in the denervated paw more than a week after the adrenaline was given;

by this time it had disappeared in the normal paw. There was ground, then, for supposing that the delay in the disappearance of the pilocarpine response after complete denervation, and the variability of the response at a certain stage, had some relation to the output of adrenaline from the suprarenal gland.

Temperature. The following experiments were carried out to determine the effect of temperature on the pilocarpine response of a denervated foot when compared with a normal foot. The results were obtained with four kittens in which the stellate ganglion had been extirpated on the right side, and in which the sweating produced at room temperature on the operated foot by 0.5 mgm. pilocarpine was as great as, or greater than, that produced in the normal foot. When the kittens were placed for about an hour beforehand and kept during the observation in the incubating chamber at 37° C., in the case of three kittens the sweating produced by pilocarpine on the normal paw was conspicuously greater than that on the operated paw, while in the fourth the same exaggerated response of the operated foot was seen as at the lower temperature. In the case of one of the three kittens the sweat produced at the two temperatures was carefully collected on pieces of dry filter paper and weighed.

TABLE VII. Exp. 5. Right stellate ganglion extirpated.

		Sweat produced by 0.5 mgm. pilocarpine	
		After 7 minutes	After 17 minutes
$T=18^{\circ}$	Operated paw	1.3 mgm.	5.0 mgm.
	Normal paw	1.5 "	6.6 "
		After 5 minutes	After 10 minutes
$T=37^{\circ}$	Operated paw	0.4 mgm.	5.3 mgm.
	Normal paw	4.5 "	10.6 "

In this case the effect of pilocarpine on the sweat glands of the normal foot and of the foot deprived of the sympathetic innervation was practically identical at room temperature; at 37° C., when the glands of the normal foot were presumably stimulated by impulses from the sweat centres, their secretion was much more profuse than that of the denervated glands, and its onset was much earlier. The relation of these observations to the use of pilocarpine for diagnosis in man is discussed below.

DISCUSSION.

The experiments recorded above in the first place confirm the conclusion of Langley and Anderson(2), and of Langley(1), that pilocarpine produces secretion of sweat by a direct action on the gland cell, and not indirectly by an action on nerve endings. This conclusion has

hitherto seemed to be at variance with other experimental evidence, and especially with the clinical observations of Horsley (12) and others, on the failure of sweating in response to pilocarpine associated with spinal lesions. Langley has already pointed out that the clinical observation of absence of sweating in the area innervated from below such a lesion in no way implicates the peripheral neurone in the action of the drug, since the postganglionic sympathetic fibres are intact in such an area, just as in the normal area, and has suggested that, owing to the distribution of the cell stations of the postganglionic fibres to the sweat glands, Horsley's observations can only be accurate in the case of certain limited lesions of the cord. The conditions prevailing in the investigation of the level of a spinal cord lesion by Horsley's method are such as to introduce two factors into the production of sweat; for the patient is warm in bed and is often surrounded by hot water bottles and blankets and covered by a hood, on the inside of which are a number of electric light bulbs. Every aid to natural sweating is thus invoked, and a very small dose of pilocarpine is then injected, the probable effect of which is merely to augment, by a peripheral action on the gland cell, the activity already excited by impulses of central origin in the area innervated from above the lesion. The condition of the patient is in some respects similar to the condition of the kitten in a room at 37° C., in which the normal paw sweated after a dose of pilocarpine earlier and more profusely than that from which the sweat nerves were absent; the area which does not sweat being cut off from central stimulation in the patient by the lesion in the cord, and in the kitten by the absence of the peripheral neurone.

The diminution in the response to pilocarpine of the sweat glands of the cat's foot after section of the sciatic nerve, described by Luchsinger (5), and observed also by Langley (1), has been confirmed for the majority of kittens in these experiments. It has been shown, however, that this change is not due to the loss of the secretory sympathetic fibres supplying the sweat glands, but to degeneration of some other fibres of the mixed nerve, which in some way control the efficiency of the peripheral circulation, as shown by the parallel loss of the vasodilator response to small doses of histamine. On this latter point my observations are not in complete accord with those published by Dale and Richards, who regarded an increased vaso-dilator response to histamine as the normal sequel to degeneration of the whole nerve supply to an organ. It would appear that the one cat, in which they tested the response at long intervals after denervation, belonged to the unusual

type in which the exaggerated vaso dilatation persists indefinitely, and that most of their experiments were made too soon after nerve section for observation of the ultimate result. My experiments bring out the further point that impairment of vaso-dilator reaction to histamine, as of sweating with pilocarpine, ultimately supervenes after complete denervation in nearly all cases. Neither impairment is due to loss of sympathetic nerves, which usually exaggerates both types of response, the suggestion is obvious that the deficient sweating is caused by the disappearance of some other fibres in the mixed nerve, in the absence of which the peripheral circulation ultimately becomes defective. As to what these other fibres are, I have no complete evidence to offer, but the following points must be regarded as suggestive.

(1) The only other fibres in the mixed nerve known to affect the blood vessels, when artificially stimulated, are the sensory fibres.

(2) The sensory fibres have long been credited with a trophic influence on the tissues. My experiments show that the loss of vaso dilatation with histamine and of sweating with pilocarpine is associated with trophic changes in the skin.

(3) Dale and Richards have given reasons for regarding the vaso dilator action of histamine as an effect on the capillaries, necessitating a good intrinsic capillary tone for its exhibition. It is only the histamine response, not the arterial dilatation with acetyl choline, which ultimately disappears after complete denervation.

From a consideration of all these points in conjunction, a suggestion emerges that, in some way, the integrity of the sensory fibres is necessary for the permanent maintenance of a normal tone of the capillaries, and therewith, as Dale and Richards have suggested, of an efficient peripheral circulation. Whether the defect of capillary tone, with the consequent impairment of blood flow, is the *cause* of the so called trophic changes, or the *effect* of some primary, more vaguely defined, trophic influence on the tissues, are alternatives between which these experiments cannot decide, the former seems the more probable and presents a possibility which is at least deserving of further investigation. Others⁽¹³⁾ have recently suggested that the so-called trophic effects may be due to changes primarily affecting the circulation, but have associated them rather with defect of sympathetic vaso motors, which, as my experiments show definitely, has no such influence.

The evidence of the effect of ether anaesthesia and of subcutaneous injections of adrenaline, in augmenting the sweat produced by pilocarpine in a limb after degeneration of the mixed nerve supply, is entirely

compatible with the view that the amount of sweating so produced depends on the tone of the capillaries. Dale and Richards found that it was impossible to produce a dilatation with histamine in a limb perfused with Ringer's solution unless both oxygenated red cells and a certain concentration of adrenaline were present, and suggested that a normal function of circulating adrenaline may be to aid in the maintenance of capillary tone. Kellaway (unpublished communication to the Physiological Society) has shown that subcutaneous injection of adrenaline can prevent the large concentration of the blood produced by histamine in cats suffering from suprarenal insufficiency. From the observations on the augmented sweating to a dose of pilocarpine produced by adrenaline, it seems that the effect of adrenaline in raising the capillary tone is produced gradually, and in the denervated limb persists for some days. It is possible that the delay in the onset of the diminished pilocarpine response, seen in many kittens after section of the mixed nerve supply, and the indefinite persistence of an exaggerated pilocarpine response after the same operation in one kitten, are related to the amount of adrenaline circulating in the blood; if this amount be large the disappearance of capillary tone, consequent on degeneration of the sensory nerve fibres, may be postponed for a certain length of time.

SUMMARY.

1. Degeneration of the sympathetic nerve supply is not followed by diminished response to pilocarpine of the sweat glands in the cat's foot. On the contrary, the sweating is often increased.

2. Degeneration of the whole mixed nerve supply to the limb is accompanied immediately by exaggeration, but usually, after a variable longer period, by great diminution of the sweating evoked by pilocarpine.

3. The changes in secretion following either sympathetic or complete denervation are closely parallel to changes in the vaso-dilator reaction of the limb to histamine. Associated with greatly diminished response of both kinds, trophic changes in the skin are observed.

4. It is suggested that all three changes are due to defective peripheral circulation, following degeneration of sensory fibres. The altered response to histamine seems to indicate that the defect is due to a failure of the normal tone of the capillary blood vessels.

5. The sweating-response to pilocarpine, depressed after complete denervation, can be temporarily restored by subcutaneous injections of adrenal. e, or by a period of anæsthesia with ether.

The majority of the aerobic operations were kindly performed by Dr. H. E. Dole, F.R.S. I wish to thank him for the generous help given at all times in this and many other directions in the course of the work.

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THE METABOLISM OF THE SALIVARY GLANDS.

II. The blood sugar metabolism of the Submaxillary Gland.

BY G. V. ANREP AND R. K. CANNAN (*Beit Memorial Research Fellow*).

(*From the Institute of Physiology, University College, London.*)

THE only direct attempts to study the blood sugar changes in a secretory organ are, so far as we are aware, those of Chauveau and Kaufmann(1) and of Asher and Karaulow(2). The former worked on the submaxillary gland of the horse and, although their analytical methods are open to criticism, they were able to show a diminution in the sugar in the blood in its passage through the active gland. Asher and Karaulow on the other hand observed an actual increase in the sugar of the venous blood from the submaxillary gland of the dog during secretion, although they found a decrease immediately succeeding the flow of saliva. Indirect evidence, afforded by studies of oxygen consumption and respiratory quotient, for the submaxillary gland(3) and for the kidney(4) point to a carbohydrate metabolism in rest which is markedly increased in activity, whilst Cohnheim(5) has shown that the secretory activity induced by sham feeding is accompanied by an increased excretion of carbon dioxide but not of nitrogen.

The results of Asher and Karaulow are open to criticism in several particulars but the essential fact which they overlooked was the concentration of blood by loss of water to the saliva. Thus in their first experiment in the two minutes necessary for the collection of 10.57 c.c. of venous blood for analysis 4 c.c. of saliva were secreted. That is to say 14.57 c.c. of arterial blood entered the gland in the same period, and its sugar content was $14.57 \times 1.34 = 19.6$ mgms. The sugar in 10.57 c.c. of venous blood was 20.4 mgms. The difference between these two figures is within the error of the method. At such a blood flow as 5 c.c. per minute it would not be expected that there would be an exchange of sugar between the gland and each c.c. of blood sufficiently large to be detected by the method used. Moreover, it seems doubtful whether, in their experiments, the blood was collected from the submaxillary gland alone. The rates of blood flow they obtained were so high as to suggest

a large admixture of extrinsic blood. Thus, in a dog of medium size a resting blood flow of 24.26 c.c. per minute was recorded and during the stimulation of the chorda this fell to 22.1 and 20.75 c.c. in one minute and on another occasion to 20.96 c.c. in 1 min. 40 secs. Such a flow is quite unique and no less unusual is the diminution in rate of flow which accompanied stimulation of the chorda. In the three experiments recorded the total saliva secreted was in each case only about 8.5 c.c. and in every case the last stimulation failed to produce any appreciable secretion. Undoubtedly the currents used for stimulation were so strong as to kill the nerve.

The limited and contradictory nature of the work done on the carbohydrate metabolism of a secretory organ suggested to us a study of the question, and for this purpose the submaxillary gland of the dog was chosen. The improved methods of blood sugar analysis whereby the dextrose in 1 c.c. of blood may be estimated with an error of less than 0.05 mgm. appeared to present the opportunity of a successful study of the subject in a small animal.

Any disappearance of sugar from the blood in its passage through the gland may be accounted for by loss in several directions. It may have (a) been retained by the gland in the form of glycogen, (b) passed into the saliva, (c) passed into the lymph, (d) been consumed by the gland.

(a) That the gland does contain glycogen was determined in the case of eight pairs of glands. The amount found varied from 0.1 to 0.4 p.c. and the two glands of any one animal agreed closely. The glands were however taken from dogs which had been submitted to a variety of experimental procedure and it is probable that the normal content is not so variable. Pflüger's method was used. The variations in glycogen content accompanying activity will form the subject of a future communication and will not be further discussed here. Whatever the fate of the sugar may be—whether it is oxidised, undergoes unoxidative disintegration or is stored in the form of glycogen—in all these cases we are entitled to call it "consumption."

(b) Carlson and Ryan (6) have found sufficient sugar in the saliva of the cat to respond sometimes to the common sugar tests. These workers obtained the saliva either by spraying the mouth with ether after a preliminary rinsing or under local anaesthesia directly from the duct by an injection of pilocarpine. The results from the former samples must be altogether discounted as sugar may well have been present in the mouth, and in every case the handling to which the animal had been

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The routine determinations made were (1) sugar of the venous blood, (2) sugar of the arterial blood, (3) hæmoglobin ratio, (4) venous blood flow, (5) flow of saliva.

The prevailing methods of blood sugar analysis are, broadly, of two kinds—titrimetric and colorimetric. Comparison of a standard method of each class usually shows a slightly lower series of values by the former than by the latter and it is probable that these lower results more closely represent the amount of actual dextrose in the blood(8). The method of MacLean(9), of which we had had experience, being simple, rapid and economical was well suited to these experiments. Duplicate estimations were made and showed the close agreement the author claims for his method.

Glycolysis. In the first three experiments each estimation was carried as far as the protein-free filtrate immediately upon the collection of the blood in order to prevent any glycolysis. We afterwards employed neutral formalin as recommended by Denis and Aldrich(10) as an anti-glycolytic agent, preserving all blood samples for analysis after the conclusion of the experiment. Although formaldehyde is a weak reducing agent it was found that no reduction of MacLean's solution by formalin was given, probably because of the low alkalinity of the reagent. Further, not only was the sugar concentration of the blood maintained over 24 hours in the presence of formalin but the concentration in the plasma was unchanged. It was therefore possible to make analyses of both blood and plasma on specimens preserved in this way. One drop of neutral (40 p.e.) formalin was added to each sample of about 4 e.e. of blood. In Table I is given an example of some of these experiments on the effect of formalin.

TABLE I. Blood sugar in mgms. per 100 c.c. blood.

	Blood alone		Blood + formalin		Blood + formalin + sugar		Blood + sugar
	Blood	Plasma	Blood	Plasma	Blood	Plasma	
I. Estimated at once	101	236	161	233	275	411	—
After 5 hours	107	152	162	233	272	411	—
II. Estimated at once	—	—	172	202	—	—	—
After 3 hours	—	—	175	197	—	—	—
After 18 hours	—	—	172	200	—	—	—
III. Estimated at once	—	—	—	—	252	—	252
After 24 hours	—	—	—	—	255	—	155

Anti-coagulant. The blood was received into dry tubes containing a very little powdered potassium oxalate. The pipette in which the venous blood was collected was likewise thinly dusted with the same powder.

however, be satisfied that the fluctuations in our values are related neither to changes in blood sugar level nor to the velocity of the blood flow through the gland. We must ascribe these irregular results to the greater error of the blood sugar determination as compared with that for oxygen. To be on the safe side one must consider, in each experiment, the range of these fluctuations.

Effect of atropine on the sugar consumption. If we compare the mean resting consumption already quoted with the mean consumption of the atropinised gland we find a close agreement.

Exp.	Resting consumption						Mean
	III	IV	VI	VIII	XI	XIV	
Before atropine	1.8	0.77	2.1	2.6	2.0	2.9	2.0
After atropine	1.6	1.7	2.9	1.7	2.1	3.0	2.2

The differences fall well within the fluctuations of the resting consumptions for each particular experiment and justify the conclusion that atropine does not affect the consumption of the resting gland.

Rate of blood flow and sugar consumption. The question of the relation of blood flow and sugar consumption will be more fully dealt with in a later communication. Here it will be sufficient to note that in Exp. II there was a rising consumption and a steady blood flow, in Exp. III a falling consumption and a rising blood flow, in Exp. IV a rising consumption and an irregularly oscillating blood flow, whilst in Exp. VI (cp. Appendix) there was a steady blood flow and a fluctuating consumption. These examples would seem to show that small variations in blood flow do not affect the consumption and that the latter may vary within certain limits when the blood flow remains steady.

Effect of pilocarpine on sugar consumption. The consumption of the gland secreting under pilocarpine was much increased, and this increase was broadly related to the activity of the gland as measured by the flow of saliva. Many examples occurred of the observation of Asher and Karaulow that the sugar concentration of the venous blood was above that of the arterial but this relation was always reversed when the correction for concentration of the blood was applied. The method of calculating was as follows:

The figures for the first pilocarpine period of Exp. VII are:

Venous blood sugar 1.65 mgms. per 1 c.c.

Venous blood flow 1 c.c. in 0.33 min. Saliva flow 0.26 c.c. in 0.33 min.

Hence 1 c.c. venous blood corresponded to 1.26 c.c. arterial blood which contained 1.26×1.57 mgms. sugar = 1.98 mgms. Hence the consumption was 0.33 mgm. in 0.33 min.

Weight of gland 5 grms. Hence consumption was 12 mgms. per gram per hour.

Results were controlled for lymph flow in the way already explained, the calculation being as follows

Plasma sugar 1.95 mgms per 1 c c Ratio of haemoglobin concentration, venous to arterial 1.32 : 1

Hence total oxidation per 1 c c venous blood was 0.32 c c

saliva " " " 0.26 c c

Therefore lymph " " " 0.06 c c

And lymph sugar was $0.06 \times 1.95 = 0.117$ mgm

1.32 c c of arterial blood corresponded to 1.00 c c venous and contained 2.07 sugar
Therefore consumption in 0.33 min was $2.07 - (1.65, 0.117) = 0.303$, i.e. 11.1 mgms per gram per hour

The above example was chosen as showing the greatest divergence that has been obtained between the results calculated by the two methods

The figures given in the Appendix are calculated without consideration of the lymph flow. This is justified on the grounds that it is not at present known whether the lymph leaving a working gland carries away sugar or whether it leaves as a sugar free fluid. In the former case the consumption recorded would be too great by the amount of such sugar escape, in the latter case the recorded concentration of the blood would be less than the actual by the amount of the lymph flow and for that reason the determined consumption would be somewhat too low. Table II demonstrates the differences in results which were obtained (a) by ignoring lymph flow, (b) assuming the lymph removes sugar in concentration equal to that of arterial blood. The difference is not of an order to affect the conclusions drawn from the experiments.

TABLE II—Exp VII Comparison of results calculated (1) without allowing for lymph flow, (2) assuming the lymph to have the same concentration of sugar as the arterial blood

Period	Haemoglobin ratio	Lymph c c per min	Consumption mgms sugar	
			Ignoring lymph	Including lymph sugar
1	100	00	2.1	2.1
2	105	01	3.6	3.4
3	100	00	2.2	2.2
pilocarpine 4	132	18	12.0	11.1
5	132	14	7.8	7.1
6	110	04	5.4	5.2
7	104	03	4.8	4.7
8	104	01	3.0	3.0
9	100	00	2.4	2.4
10	98	00	1.2	1.2
pilocarpine 11	147	19	4.2	3.6

In every case the active gland consumed more sugar than when in the resting condition. Moreover, there was a broad dependence of the

sugar consumption upon the rate of salivary secretion. A few examples will suffice (see also the illustrative experiments given in the Appendix).

Exp. I. Gland 5 grms.

	Rest		Pilocarpine		Rest
Saliva c.c. per grm. of gland per hour	—	—	4.32	3.96	—
Consumption mgms. sugar per grm. gland per hour	2.3	2.4	6.8	6.7	1.6

Exp. III. Gland 2.9 grms.

	Rest		Pilocarpine		Rest	
Saliva	—	—	6.62	8.69	—	—
Consumption	2.2	1.3	11.2	16.8	1.8	1.3

Exp. VII.

	Rest		Pilocarpine					Rest		Pilo.	Rest
Saliva	—	—	9.24	5.76	1.92	0.72	0.48	—	—	6.6	—
Consumption	2.1	2.2	12.0	7.8	5.4	4.8	3.0	2.5	1.2	4.2	0.5

If, in any one experiment, the difference be taken between the mean resting consumption per gland and the consumption per gland for a given active condition a figure may be obtained for the sugar used to produce 1 c.c. of saliva. Thus, in *Exp. II* the mean resting consumption was 0.09 mgm. per gland per min. when the gland was secreting 0.13 c.c. saliva in a minute. That is to say, 0.13 c.c. saliva was produced at the expense of $0.32 - 0.09 = 0.23$ mgm. sugar, so that the glucose used to produce 1 c.c. of saliva would be 1.8 mgms. The means of the values thus calculated in each experiment are:

I	II	III	IV	V	VI	VII	VIII	
1.0	1.8	2.7	0.7	2.2	1.1	1.2	1.0	Mean 1.5 mgms.

These show the wide variation, that would be expected in any attempt to give a quantitative expression on a loose differential basis to several unknown factors—nature of the saliva secreted, glycogen changes and time relations of secretion and consumption. Indeed, such a quantitative expression may only be used with the greatest reserve, and is here deduced merely for comparison with results from studies of oxygen consumption. Barcroft gives 0.6 c.c. of oxygen as a very approximate figure for the oxygen used in the production of 1 c.c. of saliva under the action of adrenalin. This expressed in terms of glucose oxidised gives 0.8 mgm. glucose—a figure about half of ours.

CONCLUSIONS

- 1 The resting submaxillary gland consumes blood sugar
- 2 The mean rate of blood sugar consumption varied in 15 experiments, from 0.8 to 2.9 per gram of gland per hour, being fairly constant for any one experiment
- 3 The average figure for all the experiments was 2.1 per gram per hour
- 4 Atropine does not change the blood sugar consumption of the resting gland
- 5 Pilocarpine increases the blood sugar consumption. The increase being within broad limits proportional to the rate of salivary secretion
- 6 The increase in sugar consumption per 1 c.c. of saliva secreted varied in eight experiments from 0.7 to 2.7 mgms., being also fairly constant in any single experiment
- 7 The average figure of blood sugar consumption per 1 c.c. of saliva secreted was 1.5 mgms. per gram of gland per hour

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APPENDIX.

Time	Mgms. sugar per 100 c.c. blood		c.c. per minute		Consumption per grm. per hour
	Venous	Arterial	Ven. blood	Saliva	
<i>Exp. I.</i> Wt. of gland 5.0 grms.					
0	150	180	0.65	—	2.3 mgms. dextrose
0:55'	127	160	0.63	—	2.4 "
1:34	5.0 mgms. pilocarpine injected subcutaneously				
2:00	130	134	2.14	0.36	6.8 "
3:00	123	130	1.80	0.33	6.7 "
4:00	110	121	0.38	0.07	1.6 "
<i>Exp. VI.</i> Wt. of gland 3.1 grms.					
0:0	141	152	0.73	—	1.6 "
0:12	142	160	0.90	—	3.3 "
0:21	120	138	0.75	—	2.7 "
0:35	125	138	0.70	—	1.7 "
0:45	96	109	0.78	—	2.0 "
0:50	95	109	0.78	—	2.2 "
1:20	61	67	0.75	—	0.9 "
1:30	2.0 mgms. pilocarpine				
1:35	93	99	4.0	0.44	13.5 "
1:41	92	96	1.33	0.20	4.8 "
1:47	98	95	1.00	0.15	2.4 "
1:52	1.5 mgms. atropine				
1:57	57	79	0.90	—	3.6 "
2:00	51	67	0.73	—	2.3 "
<i>Exp. VII.</i> Wt. of gland 5.0 grms.					
0:0	134	148	1.25	—	2.1 "
0:8	0.3 grm. morphia				
0:29	114	143	1.22	—	3.6 "
0:41	129	147	1.15	—	2.2 "
0:51	2.0 mgms. pilocarpine				
1:00	165	157	3.0	0.77	12.0 "
1:10	164	157	2.07	0.48	7.8 "
1:26	137	146	2.0	0.16	5.4 "
1:43	130	147	1.76	0.06	4.8 "
1:50	107	122	1.33	0.04	3.0 "
2:00	101	119	1.00	0.005	2.5 "
2:25	89	102	0.69	—	1.2 "
2:40	2.0 mgms. pilocarpine				
2:45	121	107	1.6	0.55	4.2 "
2:50	1.0 mgm. atropine				
2:55	108	112	0.93	—	0.5 "
<i>Exp. VIII.</i> Wt. of gland 4.3 grms.					
0:0	158	186	0.65	—	2.5 "
0:30	155	179	0.78	—	2.5 "
0:41	153	174	0.96	—	2.8 "
0:52	0.75 mgm. pilocarpine				
0:56	161	167	1.04	0.13	4.1 "
1:11	146	164	0.83	0.08	3.4 "
1:33	128	155	0.75	0.03	3.3 "
1:47	1.25 mgms. pilocarpine				
1:49	147	155	5.9	0.70	21.0 "
2:23	98	139	0.74	Just ceased	4.2 "
2:41	1.25 mgms. pilocarpine				
2:43	128	125	2.05	0.7	11.2 "
2:53	123	149	1.5	0.5	7.2 "
2:58	1.0 mgm. atropine				
3:03	102	125	0.66	—	1.7 "
3:34	114	137	0.61	—	1.7 "

SALT AND WATER ELIMINATION IN MAN.

By M. M. BAIRD AND J. B. S. HALDANE, M.A.

(From the Laboratory, Cherwell, Oxford)

THE following experiments were undertaken to investigate the retention of water which one of us had found to occur when it is taken after a strong salt solution. The subject was J. B. S. H. (95 kilos.), but similar

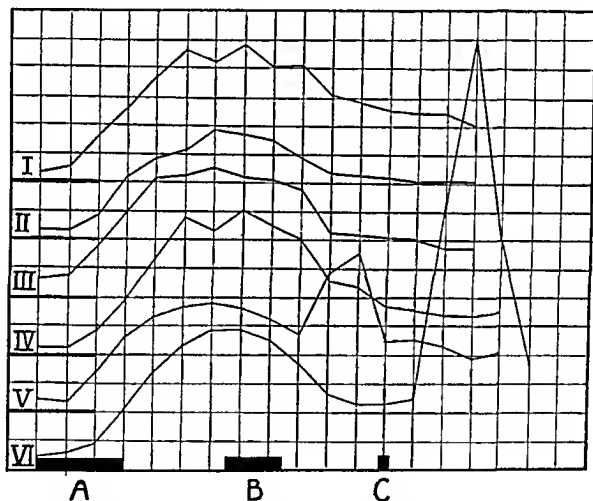


Fig 1 Units of abscissa Time in half hours Units of ordinate. Rate of urine secretion in c c per hour One division = 50 c c The zero line of each curve is shown below it on the left At A, 29.25 gm NaCl + 12.6 gm NaHCO₃ were taken in 500 c c of water in each experiment. At B, the following quantities of water in litres were drunk: I, 0, II, 0.5, III, 1.5, IV, 2.0, V and VI, 2.5 At C, 0.5 litre was drunk in Exp VI only

results were obtained on M. M. B. The salt ingested was either 38 grams NaCl, 29.25 gm. NaCl + 12.6 gm. NaHCO₃, which contains the same number of molecules, or a mixture in which K, Ca and Mg were present

is needed to dilute it to isotonicity. It may be largely combined with, or adsorbed by, colloids. According to Padtberg⁽⁴⁾ the skin and subcutaneous tissues are the chief place of storage. The process of storing is a fairly slow one, and until it is accomplished there is an excessive salt concentration in the blood. The first diuresis is due to this surprise effect, and comparable with alimentary glycosuria. When, however, the majority of the salt has been stored it is not readily liberated again into the blood; and the effect of large water ingestions is partly a water diuresis and partly oedema of the depot tissues, whilst the excretion of salt is very slow. The slowness both of storage and release of the salt can, we think, be accounted for by the relatively small proportion of the blood normally passing through the skin and adjacent connective tissues. The capacity of certain tissues for storing salt apart from water probably explains the fact that salt solutions were best retained when somewhat hypertonic.

SUMMARY.

The diuresis produced by drinking hypertonic salt solutions is independent, within wide limits, of the amount of water ingested. Salts are less mobile within the body than water.

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OBSERVATIONS ON AUGMENTED SALIVARY SECRETION. BY G. V. ANREP.

(From the Institute of Physiology, University College, London.)

IN 1889 Langley described a peculiar effect of stimulation of the cerebral secretory nerves of the salivary glands upon the amount of saliva obtained by stimulating the sympathetic nerve. According to his observations made on the dog, the sympathetic nerve when it is stimulated shortly after the cranial nerve, produces a brief rapid secretion from any or all the salivary glands. A very brief stimulation of the cranial nerve is sufficient to produce this effect. The augmenting effect disappears in time, although the sympathetic is not stimulated in the interval. If the sympathetic is stimulated for a longer time or several times in succession the effect is found to disappear quickly, and, as a rule, the third or fourth stimulation is already ineffective. Atropine in doses sufficient to paralyse completely the cranial nerve paralyzes its augmenting action. Direct measurements of the blood flow through the gland as well as the fact that atropine paralyzes the augmenting action of the cranial nerve led to the conclusion that mere vascular dilatation is not the cause of the augmented secretion. Langley explains it by a transitory increase in the irritability of the gland to impulses reaching it by the sympathetic nerve.

In the course of a series of experiments with the salivary glands performed for a different purpose I had frequent opportunities to observe the facts described by Langley. And my observations were in every detail in complete accord with Langley's. In this communication only those experiments are described which deal with the explanation of the augmented secretion and which suggest that its cause is probably different from the one advanced by Langley.

The effect of filling the ducts of the salivary gland. All experiments were performed under morphia and C.E.' mixture or under chloralose, the latter being injected intravenously in doses 0.075 or 0.1 per kilo.

Exp. 1. Dog 8, 2 kg. Chloralose Cannula in the right submaxillary duct. The right chorda tympani and the right vago-sympathetic nerve cut and prepared for stimulation. The salivary cannula is joined with a glass tubing of narrow bore fixed to a millimetre scale, each 45 mm. of the tubing being equal to 0.2 c.c. The secretion is noted every 30 seconds unless otherwise mentioned.

On several occasions the stimulation of the atropinised chorda was tried but this was not found to have any effect on the rate of the perfusion of the gland.

Exp. 4. Dog 9, 4 kg. Chloralose. Preparation as in Exp. 2. The level of the saliva in mm. is noted every 30". Eight mgm. of atropine were injected half an hour before the first reading. The chorda was completely paralysed. Tube horizontally, saliva stays for 8 min. at 320 mm. Tube placed vertically—saliva slowly runs down into the gland and soon takes a new level at 280 mm. No movement of the saliva for 2 min.

(1) 4.45. 280, 280, sy. 30" (c=12) 298, 296, 285, 280, 280.

(2) 4.57. 280, 280, sy. 30" (c=11) 301, 302, 298, 283, 280, 278, 278.

(3) 5.5. 278, 278, sy. stim. at figures in thick type (c=12) 301, 301, 303, 300, (c=11) 312, 310, 315, —, —, 300, 316, 310, 305, 294, 285, 285.

Some saliva ran out, new level at 222 mm.

(4) 5.22. 222, 222, sy. stim. for 5 min., the maximal level is reached (c=11) in 2 min. at 254. Stimulation stopped—saliva falls and in 5 min. reaches 222. Massage of the gland—maximal level 275, massage stopped—saliva down to 222. Massage of the gland, saliva rises to 275, prolonged stimulation of the sympathetic directly after the massage—saliva down to 256 where it remains stationary during the stimulation. In 2 min. the stimulation is stopped and the saliva soon comes back to 223.

Exps. 3 and 4 indicate that the stimulation of the sympathetic nerve produces a diminution of the capacity of the ducts of the submaxillary gland. In Exp. 3 with the perfusion of the gland it caused a diminution of the flow of the fluid through the gland. In Exp. 4 it developed a pressure within the ducts. The last experiment cannot be explained by a sympathetic secretion as no progressive rise of the saliva was observed with each successive stimulation.

Massage of the gland. Massage of the submaxillary gland after a previous chorda stimulation has a variable effect. At the beginning of an experiment only very little saliva can be pressed out. If some saliva is pressed back into the gland it likewise cannot be pressed out by massage. The effect of massage does not alter after protracted stimulation of the chorda so that the retention of the saliva cannot be due to blocking of the ducts by a viscid fluid. In some dogs, however, especially in those which were kept for a long time under anæsthetic, the effect of massage became more and more marked and a large amount of saliva could be pressed out of the gland after every chorda stimulation. Saliva which has been forced into the gland does not stay in it, but slowly flows out, and by means of some mechanical pressure on the gland it can be completely pressed out. The augmented secretion does not disappear and in some experiments it is still larger than the effect of massage, in others, massage has been found more effective than the stimulation of the sympathetic.

Exp 5 Dog 8, 5 kg Preparation as in Exp 1, but the secretion is recorded in drops
(1 c c = 70 drops)

No of drops			No of drops		
(1) 4 55 chorda	60"	13	(2) 5 22 chorda	60"	12
interval	30	2	interval	90	4
massage	60	11	v symp	60	19
v symp	60	9	interval	30	1
massage	60	1	massage	60	$\frac{1}{2}$
(3) 5 22 massage	60	2	(4) 5 36 chorda	45	11
interval	,	0	interval	120	3
massage	"	2	v symp	60	14
interval	"	0	interval	"	2
v symp	"	1	massage	"	1
interval	"	0	interval	"	0
v symp	"	2	v symp	"	1
interval	"	0	interval	"	0
(5) 5 55 chorda	45	12	massage	"	2
interval	60	3	(6) 6 12 chorda	45	12
massage	60	10	interval	120	3
v symp	90	13	v symp	90	24
massage	60	0	massage	60	1

In this particular experiment the stimulation of the sympathetic nerve produced a bigger effect than massage. In several other experiments, however, the action of the sympathetic was much weaker than massage. In those experiments the augmented secretion was also very weak. Exp 5 indicates that during the augmented secretion there is only an emptying of the ducts from stagnant saliva but not an actual secretion. The amount of saliva obtained from the gland by massage with a following stimulation of the sympathetic is approximately the same as the one obtained by the stimulation of the sympathetic without a previous massage. In the above experiments the figures are 16, 20 and 23 drops as against 14, 19 and 24 drops. In each case the stimulation of the sympathetic was continued much longer than the secretion so that a still longer stimulation would not cause any more secretion. Massage of the gland after a sympathetic stimulation does not cause any flow of saliva, which suggests that the gland is empty.

Two experiments were performed with the use of ergotoxine, in the first ergotoxine was injected intravenously, in the second the gland was perfused with a solution of ergotoxine in gum saline. The blood flow through the gland was recorded at the same time as the secretion. In both experiments it was found that the augmented secretion was paralysed with far smaller doses than the vaso constrictors. The experiments with ergotoxine support Langley's statement that the augmented effect is independent of the vascular condition of the gland. Langley found the augmented secretion in the parotid gland of the dog and in the sub-

maxillary of the cat to be much less marked. In accordance with this a few experiments performed with perfusion of those glands showed a much smaller effect of the sympathetic on the rate of flow of the fluid through the gland as compared with the one described for the sub-maxillary gland.

CONCLUSIONS.

1. No support is given to the explanation of the "augmented secretion" being due to an increased irritability of the salivary gland to impulses reaching it by the sympathetic nerve.

2. The suggestion is put forward that the augmented effect of the sympathetic is an emptying of the gland of stagnant saliva caused by a narrowing of the ducts either due to their own contractility or due to a contraction of tissues around the alveoli and the ducts.

3. The most probable explanation of the disappearance of the augmented effect is the absorption of the saliva from the ducts and alveoli.

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THE EXCRETION OF CHLORIDES AND BICARBONATES BY THE HUMAN KIDNEY. BY H. W. DAVIES, M.B., B.S., J. B. S. HALDANE, M.A. AND G. L. PESKETT, B.A.

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AMBARD and PAPIN(1) showed that in any individual, man or dog, there is a definite maximum to the possible concentration of urea in the urine; that this concentration can easily be reached; and that it is, to a considerable extent at least, independent of the concentration of chlorides simultaneously excreted. They also brought forward some evidence to show that the limiting concentration for chloride in man is between .3 N and .4 N, while in the dog, according to Bailey and Bremer(2) it is about .17 N. Davies, Haldane and Kennaway(3) have shown that the maximum attainable concentration of bicarbonate is of the same magnitude. The experiments here recorded were made to determine the relations between the various concentrations which are possible in the urine at the same time.

All experiments but one were made on J. B. S. H. (weight 95 kilos.). Chlorides were estimated by Volhard's method, bicarbonates with J. S. Haldane's(4) blood-gas apparatus, phosphates with uranium acetate and cochineal, and urea by Krogh's(5) or Marshall's(6) method. Concentrations are expressed in terms of normality, or for phosphates molarity. Volumes are given in c.c., weights in grams.

When strong solutions of NaCl were drunk the urinary chloride concentration rose rapidly to a value varying between .29 N and .33 N, the value being independent of the volume excreted per hour, and only rising slightly when more salt was taken. Table I gives the results of a typical experiment. Here 18 grams of NaCl in 200 c.c. of water were drunk at 13 hours, and again at 16.15.

TABLE I.

Time	10-12	12-13	13-14	14-15	15-16	16-17	17-18	18-19
Volume per hour ...	41	43	78	154	162	115	181	211
Chloride concentration	.201	.259	.300	.304	.312	.331	.328	.324

The limit of .330 N was only passed during extreme thirst, the highest value recorded being .338 N. The maximum did not vary in J. B. S. H.

during 18 months, but higher values were found in two other healthy men, lower in one. Out of 70 bicarbonate concentrations determined on J. B. S. H. only one exceeded .330 N. In this case the value recorded, .358 N, is probably erroneous. Values higher than .320 N were, however, reached on several occasions. The maximum molecular concentrations of chloride and bicarbonate are therefore practically identical.

When chloride and bicarbonate were taken together or successively both appeared in the urine in large amounts. Neither reached its maximum concentration, but the sum of the two reached a value which (expressed in normality) was equal to the maximum of either. Thus, in the experiment summarised in Table II, 26 grams of NaCl had been taken on the previous day, and 13 at 9.30. Ten grams of NaHCO₃ and three of NaCl were taken in 150 c.c. of water at 11.10, 12.10, 13.10, 14.10 and 15.10, also a little extra water at 13.45 and 16.00.

TABLE II.

Hours ending	Volume per hr.	Cl	HCO ₃	Cl + HCO ₃	CON ₂ H ₄	Δ
11.40	62	.321	.000	.321	.252	1.82°
12.40	152	.246	.063	.309	.186	—
13.40	221	.185	.134	.319	.135	—
14.40	391	.150	.158	.308	.117	—
15.40	294	.141	.177	.318	.089	1.40°
16.40	335	.138	.171	.309	—	—
16.40-18.30	> 200	.143	.190	.333	.101	—

Here the effect of the bicarbonate was to lower rapidly the chloride concentration, though more chloride was being ingested than excreted; but the sum of the concentrations remained steady within 8 p.c. It is noticeable that this value was quite independent of considerable variations in the concentration of urea or the depression of the freezing point.

If bicarbonate be given without chloride the latter may almost disappear from the urine. In the experiment recorded in Table III, 25 grams of NaHCO₃ were taken in 100 c.c. of water at 10.45, and

TABLE III.

Time	Volume per hr.	Cl	HCO ₃	Cl + HCO ₃	CON ₂ H ₄	Gms. NaCl per hr.
10-11	39.5	.212	.003	.215	—	.489
11-12	55.5	.134	.135	.269	.420	.435
12-13	105.5	.078	.209	.287	—	.481
13-14	169	.043	.258	.301	.135	.425
14-15.05	155	.024	.269	.293	—	.217
15-05-16	123	.026	.280	.306	.193	.187
16-17	134	.072	.242	.314	—	.564
17-17.30	143	.082	.220	.302	.178	.684
17.30-18	95	.090	.201	.291	—	.500
18-19	142.5	.085	.201	.286	.213	.708
19-20	144	.088	.208	.296	—	.740
20-21	134.5	.095	.146	.241	.202	.747

20 grams of NaCl with 5 of NaHCO_3 in 400 c.c. between 15.20 and 16.15. A litre of water was drunk at 17.00 and again at 19.00.

Here less salt was taken, so the maximum was lower. At first the chloride output was unaffected, but it fell sharply when the sum of chloride and bicarbonate reached .30 N. In presence of the bicarbonate even 20 grams of NaCl failed to raise the urinary chloride concentration to its normal value. Four other experiments gave results like those of Tables II and III. The maximum value of $\text{Cl} + \text{HCO}_3$ reached was .334 N on two occasions.

The antagonism between chloride and bicarbonate excretion also appears when the bicarbonate is being excreted as a result of forced breathing. In an experiment where H. W. D. over-breathed for 87 minutes, his mean alveolar CO_2 being 1.67 p.e., the urinary bicarbonate rose to .053 N, while the chloride fell from .120 N to .015 N, although the rate of water excretion was not doubled. This fall is the more remarkable since removal of CO_2 slightly increases the chloride content of the plasma.

Not only is a simultaneous excretion of urea without effect on the kidney's capacity for concentrating chloride and bicarbonate, but urea ingestion, though it may lower the chloride concentration by promoting diuresis, considerably increases the output per hour. Thus, after taking 100 grams of urea the chloride output rose from .88 gram NaCl per hour to values which exceeded 1.2 grams per hour during five consecutive hours. On then taking 20 grams of NaCl, as in Table III, the chloride output at once rose to 2.4 grams per hour as compared to 0.75.

Further experiments were undertaken to determine whether the constancy of the maximum of $\text{Cl} + \text{HCO}_3$ was due to the existence of a maximum possible concentration in the urine of Na or total cations. Attempts made to increase the chloride concentration by taking NH_4Cl failed, owing to the vomiting caused by strong solutions. The question was, however, settled by simultaneous ingestion of chloride and acid phosphate. The following were ingested:

24.15. 20 gm. NaCl + 500 c.c. water.

9.00. 16 gm. $\text{NaCl} + 500$ c.c. water.

12.05. 20 g. $\text{NaCl} + 220$ c.c. water.

13.05. 20 g. $\text{NaCl} + 220$ c.c. water.

16.05. 500 c.c. water.

17.05. About 1 litre tea.

19.15. About 1 litre various fluids + dinner.

The urines produced are shown in Table IV.

Though some of the salts were lost through diarrhœa the chlorides were but little depressed by the phosphates, and the sum of the two rose

TABLE IV.

Hours ending	Volume per hr.	Cl	H ₂ PO ₄	CON ₂ H ₄	Cl + H ₂ PO ₄
11	124	·330	·0048	·184	·335
12	145	·338	·0038	—	·342
13	142	·329	·0120	—	·341
14	165	·308	·0397	·160	·348
15	139	·294	·0696	—	·364
16	131	·293	·0809	·147	·374
17	129	·310	·0718	·151	·382
17-18.32	96	·312	·0795	·181	·392
18.32-20.32	77·5	·303	·0662	·221	·369

to a far higher value than was ever obtained for chloride, bicarbonate, or both together. Moreover, it reached a maximum at a time when there was little thirst, which always accompanied very high Cl + HCO₃ concentrations. Hence the property which causes chlorides and bicarbonates to share a common maximum is neither that they share a common cation, nor that both are ionised.

DISCUSSION.

The fact that the Cl + HCO₃ of the urine has a definite maximum which is unaffected by the urea or phosphate content of the urine or its total molecular concentration suggests strongly that the former salts are concentrated by a different part of the kidney from that which concentrates the urea, phosphates, and presumably other no-threshold bodies. This view is borne out by the fact that the dog can concentrate urea to 1·6 N, but its maximum for chlorides is apparently only ·17 N. The limit is more probably set by the difference in salt concentration between plasma and urine than by the absolute concentration in the latter. Since J. B. S. H.'s normal colloid-free plasma contains about ·115 N chloride and ·025 N bicarbonate, or ·14 N in all, this difference is about ·17 N. The fluctuations in the maximum may then be explained as due to changes in the salt content of the plasma. It is clearly indifferent to the concentrating cells whether the difference in concentration is due to Cl or HCO₃. The factor which limits their performance is presumably the osmotic leakage of water from the plasma into the concentrated urine, possibly a leakage of cations.

Our results are quite consistent with Heidenhain's (7) theory that the glomerulus alone is responsible for the secretion of "water and those salts which everywhere accompany water in the organism." On this view the maximum is a measure of the glomerular concentrating power.

If, however, we consider that the urine is concentrated by the tubules, we observe that chloride and bicarbonate agree with one another and water, and differ from all the anions so far studied, except bromide, in

all the following properties: (1) they are present in large amounts in the plasma; (2) they possess high thresholds for the kidney; (3) their excretion is more interfered with than that of other urinary constituents by partial obstruction of the ureter [Cushny(8)] or renal artery [Marshall and Crane(9)]; (4) their excretion is less interfered with than that of any other urinary constituent by a short asphyxia of the kidney [Marshall and Crane(9)]. The last two facts are most easily explained on the view that all or most of the water, chloride and bicarbonate leave the blood by filtration through the glomerulus, while most other substances are in part actively excreted by the tubules. If this is the case bicarbonate must be reabsorbed from the filtrate under normal conditions, water whenever the urinary chloride or bicarbonate is higher than that of the plasma, and chloride when the urinary chloride is lower than that of the plasma. It may be that all are absorbed at once in constant proportions, as on Cushny's(10) theory. If then the glomerulus is a filter the chlorides and bicarbonates are concentrated by a process of reabsorption, and the observed maximum is a measure of the limit to which this concentration can be carried in face of the tendency to osmotic diffusion of water in the opposite direction.

But if the no-threshold bodies are also concentrated by reabsorption we should expect them to hinder the concentration of chloride and bicarbonate, which is not the case. And this hindrance would also occur if the no-threshold bodies had all been excreted into the tubules before the concentration of the chloride and bicarbonate. For the urea in the tubules would tend to hold back water from the reabsorbing cells. Hence excretion must take place lower down the tubules than reabsorption. It is true that Ambard and Papin found that the urinary chloride did not affect the urea maximum of the dog. But as the chloride concentration of the urine never exceeded that of the plasma in those of their experiments where the urea maximum was reached, this result was to be expected. Our experiments, therefore, are in harmony with Metzner's(11) view that both reabsorption and excretion occur in the tubules.

SUMMARY.

1. There is a maximum possible molecular concentration of chlorides in the urine. For J. B. S. H. this is about .33 N.
2. The maximum for bicarbonates has the same value.
3. When chlorides and bicarbonates are being excreted together the maximum possible sum of their molecular concentrations has this same value.

4. This maximum is independent, within wide limits, of the total molecular concentration of the urine, or those of urea and phosphates.

5. Chlorides and bicarbonates must be concentrated by the same part of the kidneys, which is probably reabsorptive, urea and phosphates a different one, which is excretory.

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ON THE INFLUENCE OF CHANGES OF CONCENTRATION OF THE H^+ RESP. OH^- IONS ON THE LIFE OF THE TISSUE CELLS OF VERTEBRATES.

I. The influence of temporary changes of reaction of the medium.

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It has been shown by many observers that variations in the reaction of blood and lymph are slight in normal conditions and inconsiderable even in pathological cases of "acidosis". It appears that any marked change of the reaction in higher animals is incompatible with life. It is not, however, known whether death caused by a change in the reaction of the blood is due to an action on a few particularly sensitive organs or to a detrimental influence on the life of all the cells. In order to investigate this question we have used the method of tissue cultures (explantation¹), since this allows the influence of the reaction of the medium to be determined on such fundamental properties of the cells as growth and division.

Methods Our observations have been made on the spleen of the rabbit. We selected this because its cultivation outside the organism gives very good and uniform results, and because the cells of the spleen during their life in the organism are surrounded by a relatively constant internal medium. Moreover, tissue cultures of the spleen have been studied in cytological as well as histological respects, by Maximov⁽¹⁾ and others. The object of the observations was to determine the concentration of H^+ and OH^- ions compatible with life.

As acid solutions we employed lactic acid "buffer" and acetic acid "buffer", as alkaline solutions the "borate mixture" of Sorensen⁽²⁾, and the "ammoniacal mixture" of Michaelis⁽³⁾. We made up these mixtures so that they were in general isotonic with the fluids of the body and that the concentration of the salts in them corresponded to $\frac{1}{2}N$. The primary strong solutions of acids and alkalis were taken to be practically free from microbes and left unsterilised, all farther manipula-

¹ For a bibliography, a survey of the data obtained by this method, and an account of the technique, see Krontovski and Poleff *a Method of Tissue Cultures*, Pts I and II, 1917 (Russ.)

tions were strictly aseptic, according to the usual bacteriological technique; as a matter of course the vessels, pipettes, distilled water, etc. were sterilised beforehand. The concentration of the H ions of each mixture was in every case determined after the experiment by "gas-chains," by means of the compensation method of Poggendorf and Du Bois-Reymond, using the electrodes of Michaelis and Hasselbalch (in their last modification) and as another electrode, the $\frac{1}{10}$ N calomel electrode.

A series of regulating mixtures (buffers) were prepared, containing an increasing concentration of H ions. In a large quantity of each, not less than three small pieces of spleen, about .5 mm. in diameter, were placed for half an hour at room temperature. They were then washed with Ringer's fluid, and placed upon the glass cover of a Gabritcheffski dish, and immediately covered with a drop of rabbit serum. Then all, at the same time and from the same drop of rabbit's plasma, were covered with a drop of the plasma, and the dishes with the cultures placed in a thermostat at 38° C. The plasma was obtained by centrifuging in paraffined and refrigerated test tubes. The mixture of serum and plasma forms, as has been shown by one of us (4), a medium favourable to the growth of the tissue. The control pieces were placed in Ringer's fluid for half an hour instead of in a mixture, otherwise they were treated in the same way.

In all, more than 400 cultures were made. The general results only are given here and cytological details omitted.

The degree of growth is indicated in the tables by the signs in column 3.

A minus sign (—) indicates that in 10–12 days there were no cells at all on the periphery of the piece, and that there was no amœboid movement or other sign of life in the tissue itself.

A cross indicates that there was some growth in the culture, and the degree of growth by one, two or three crosses. + indicates growth of fibroblasts and × indicates that of lymphocytes and polyblasts. These indications are of course only of relative value and give the results in a very rough manner. With regard to the individual fluctuations in one and the same experiment a certain idea may be gathered from Fig. 3, p. 280, giving the results obtained with one culture.

Growth in control cultures. In the control cultures there appear generally after a few hours on the periphery of the piece, a great number of cells migrating into the surrounding medium. They consist of lymphocytes (Fig. 1, *l*) and reticular cells transforming themselves into hypertrophic polyblasts (Fig. 1, *p*) (Maximov (1)); the cells multiply by mitotic

division. On the 2nd-3rd days, fibroblasts begin to grow in the surrounding medium (Fig. 1, *f*), for the most part of the typical fusiform (or branched) shape. On the 5th-7th days, a broad zone forms itself around the piece, consisting of a vast number of wandering elements, the number of fibroblasts varying considerably.

Experiments with acid mixtures. The general results of the experiments with lactic mixtures are put together in Table I.

TABLE I. Experiments with lactic mixtures.

Mixtures	pH	Growth of the cultures	
1	5.64	+++	xxx
2	5.29	+++	xxx
3	4.97-4.89	+++	xxx
4	4.77-4.74	+++	xx
5	4.44-4.42	+++*	x
6	4.15-4.04	+(-)†	
7	3.68	-	
8	3.53	-	
Control	—	+++	xxx

* In one experiment after the action of this concentration and the following no growth at all was to be observed.

† At this concentration a growth of fibroblasts was to be observed in about one-half of the cultures; wandering cells were to be seen in one case only.

The results given in this table show that the most acid mixture, which after half an hour's action, allowed any growth (the growth of a small quantity of fibroblasts) was the mixture No. 6, in which the concentration of the H ions corresponded to pH; with this concentration, about one-half of the cultures showed some signs of growth. A con-

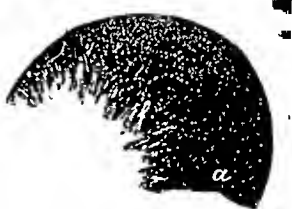


Fig. 1. Three days' control culture of spleen. Migration of a great quantity of various wandering cells (*l*—lymphocytes, *p*—polyblasts) and growth of fusiform fibroblasts (*f*).

Fig. 2. A 10 days' culture obtained from spleen piece exposed to half an hour's action of a lactic mixture with pH=4.44. Growth of fibroblasts alone (*f*), complete absence (*a*) of migration of wandering cells.

centration of the H ions about twice as strong, viz. pH 3.68, affected the cells so that afterwards they did not show any signs of life. It appears also that a mixture with pH 4.89 does not in half an hour affect in any perceptible degree the ensuing growth of the cultures. The influence of stronger concentrations of the H ions manifests itself first of all in that the wandering cells migrate from the piece later and in less quantity. After concentrations approaching the limit (*e.g.* after mixtures with pH 4.04 and 4.15, or after pH 4.44, if at pH 4.15 death ensued) this migration is, as a rule, not to be observed during the first 2-3 days (see portion *a* on Fig. 2) and the borders of the piece remain sharply outlined and only on the 3rd-5th days (sometimes, however, a little earlier) there begin to grow on sundry points of the periphery of the piece fusiform (or ramified) fibroblasts, which often attain considerable growth in one definite place, being shaped like a fan (see *ff*, Fig. 2).

Thus the cultures of spleen pieces which have been exposed to the influence of a considerable concentration of the H ions, differ markedly from the control cultures. Round the latter there are a vast quantity of wandering cells. It is evident further that the fibroblasts are more resistant to the increase of concentration of the H ions than the wandering spleen cells (lymphocytes, reticular polyblasts, etc.).

Wishing to ascertain whether solutions would exert exactly the same influence on spleen cells, if the same concentrations of the H ions were attained by means of other acids, we made a series of experiments with acetic mixtures ("buffers"). The results are given in Table II.

TABLE II. Experiments with acetic mixtures.

Mixtures	pH	Growth of the tissue cultures	
1	6.33-6.25	+	+
2	5.93-5.90	+	+
3	5.63-5.60	+	+
4	5.33	+	+
5	5.07-5.05	-	(+)*
6	4.75	-	-
7	4.42-4.40	-	-
8	4.12	-	-
Control	—	+	+

* In one case at this concentration growth was observed.

From Table II it appears that the influence of slightly acid solutions (*e.g.* No. 1) does not in any perceptible degree affect the ensuing growth of the cultures; the application of strong concentrations of the H ions is followed by an inhibition of the migration of wandering cells (so that only fibroblasts can grow) and after the action of mixtures with a still more considerable concentration of the H ions there appear in the pieces

no manifestations of life at all. In all these respects the results with acetic mixtures correspond to those with lactic mixtures. A difference, however, clearly appears in quantitative respect, if we compare the numbers (cf. Tables I and II), denoting the concentrations of the H ions which correspond to one and the same biological action on the spleen cells. The maximum concentration of the acetic mixture compatible with the life of the cells generally appeared to be that corresponding to pH 5.33 (more rarely 5.05) whilst in the experiments with lactic mixtures it corresponded to pH 4.04 (No. 6, Table I). An acetic mixture in which the concentration of the H ions was about $C_{11} = 10^{-5}$ (e.g. No. 5, Table II) exerted for the most part a destructive influence on all cells (except in one experiment where, after the action of a mixture with pH 5.05, a slight growth of fibroblasts was observed), whilst after the action of a lactic mixture with the same concentration of the H ions (e.g. No. 3, Table I) the spleen pieces showed a very good growth followed by the migration of a great quantity of wandering cells and the growth of fibroblasts.

For controlling the results we made several experiments with the action of the "buffers" mentioned, on pieces of one and the same spleen under otherwise the same conditions. In this case also the difference of action of the lactic and acetic mixtures with equal concentrations of the H ions was clear. From the results it appeared that the action of the mixtures on spleen cells depends not only upon the concentration of the H ions, but also upon other factors.

Experiments with alkaline mixtures. In order to examine the influence of alkaline reaction we made experiments (analogous to those above-mentioned) with the "borate mixture" of Sørensen (2). The results are given in Table III. It may be mentioned that under the action of the

TABLE III. Experiments with alkaline "borate mixtures."

Mixtures	pH^*	Growth of the tissue cultures	
1	8.75	+++	xxx
2	9.05	+++	xxx
3	9.6	+++ (+ +)†	xxx (xx)
4	9.78	++	++
5	10.01	++	xx
6	10.28	+	x
7	11.0	—	
8	11.15	—	
Control	—	+++	xxx

* Whilst the alkaline mixture was standing in Petri dishes, the concentration of the H ions in it became somewhat altered, so that measurements made after the end of the experiment showed a somewhat less alkaline strength, than it had had during the experiment.

† About one-half of the cultures showed a growth like the foregoing (+++), the other half, less (++).

alkaline solutions with a pH of 11 and upwards, the spleen pieces, as was observable even with the naked eye, lost their usual shape and were transformed into slimy clumps resolving into threads.

The most alkaline solution, after the action of which a growth of cultures was to be observed, was that with pH 10.28. With this concentration, though the pieces seemed altered and somewhat slimy, a rather large number of wandering cells and fibroblasts showing vital activity was to be observed. Probably the cells are capable of enduring the temporary influence of even a somewhat stronger alkaline medium. The results show that under the (temporary) influence of a medium, the alkaline strength of which is near that which finally destroys the tissue of the spleen, the cells are still capable of preserving life. Special experiments for ascertaining whether slightly alkaline mixtures exert a favourable influence on the cultures we have not as yet made.

Apart from the experiments with a "borate mixture" we also made experiments with the "ammonia standard solutions" of Michaelis. On the whole it appeared that the ammonia mixture exerts a decidedly inhibitory influence on the cells of the spleen in much less alkaline solutions, than the corresponding solutions of the borate mixture.

DISCUSSION OF RESULTS.

From the foregoing results it follows that the spleen cells are capable of preserving their life outside of the organism at a considerable (temporary) deviation of the reaction of the surrounding medium from the neutral point in the direction of an increase in acidity as well as in the direction of alkalinity. This is also illustrated in Fig. 3. The

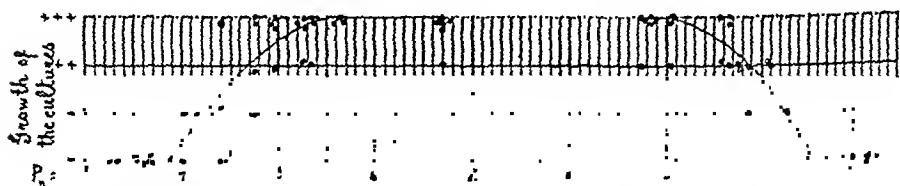


Fig. 3. The growth curve of tissue cultures after half an hour's action of various concentrations of the H ions.

capability of the fundamental vital processes of these cells to withstand a change of reaction is in striking contrast to the delicacy with which the functional action of various differentiated cells of vertebrates—*e.g.* those of the heart and the respiratory centre—respond to a change of reaction.

The maximum concentration of H ions (after temporary action)

compatible with the life of the spleen cells appeared to be pH 4.04 and the minimum pH 10.28. In the organism of higher animals with its sensitive mechanism for regulating the reaction of the internal medium such fluctuations never occur. According to Pechstein(5), muscles fatigued by stimulation have a pH of 6.84. Michaelis(3), in cases of diabetes found the pH shortly before death to be 7.11. Even intravenous injection of acids in animals according to Szili(6) increased the acidity of the blood immediately before death only to a pH of 5.9, and Radzimirski, in some unpublished experiments on the injection of alkalis, found the pH of the blood just before death to be 8.0 to 8.15. It is clear then that such fluctuations of the reaction of the blood as may occur in physiological and pathological conditions cannot kill the spleen cells.

Of all the spleen cells the greatest power of resistance against the action of acid solutions was shown by the fibroblasts. They endure the action of such concentrations of the H ions as completely inhibited the vital activity of the wandering cells. The fibroblasts appeared as the most capable of life also under other conditions, as shown by the experiments of one of us(1), they can still grow after half an hour's action of a 5 p.c. and even 10 p.c. solution of $NaCl$, and are not killed by immersion for half an hour in twice distilled water.

The experiments which have so far been made on lower animal and plant organism show that death ensues after treatment with solutions having a pH ranging in the different cases from about 4 to 5.27. The spleen cells, as we have seen, die after half an hour's treatment with a buffered mixture of pH 4.04. It is interesting to note that the limit of concentration of H ions for higher vertebrate tissue cells (spleen cells) living in an internal medium, is much the same as that for lowly organisms living in an external medium, and that both can endure, at least temporarily, considerable variation in the reaction of the medium in which they live.

SUMMARY

Cultures of small pieces of spleen of the rabbit in buffered mixtures were made outside the body. The experimental pieces were placed for half an hour in a mixture with a given H ion concentration. The control pieces were left for the same time in Ringer's fluid. Then all were placed in a mixture of serum and plasma. It was found that

1. The maximum concentration of H ions in lactic acid mixtures which left the cells still alive and capable of growth was pH 4.04, with acetic acid mixtures it was pH 5.33 (in one case 5.05). This difference

in action shows that the influence of the mixtures depends, not only on the H ion concentration, but also upon other factors.

2. The minimal concentration of H ions compatible with the life of the cells was $pH\ 10.28$.

The results show that the lymphocytes, reticular cells and fibroblasts of the spleen retain life when the reaction of the surrounding medium undergoes considerable change either to the acid or to the alkaline side—a degree of change which does not occur in the body either in physiological or pathological conditions.

3. The different spleen cells are not affected to the same degree by changes in the H ion concentration. The fibroblasts withstand an increase of H ion concentration which completely inhibits the vital activity of the wandering spleen cells—lymphocytes, reticular polyblasts, etc. Thus it is possible to obtain a pure culture of fibroblasts.

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ANOXÆMIA AND THE ADMINISTRATION OF OXYGEN. BY N MORRIS, MD

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It has long been known that in conditions where the pulmonary mechanism is interfered with there is a deficient supply of oxygen to the tissues as is manifested clinically in cases of lobar pneumonia by the appearance of cyanosis. The objects of the present research were (1) to determine the variations in the oxygen content of the blood in varying degrees of interference with the pulmonary ventilation, (2) to ascertain to what extent the oxygen content of arterial blood might be decreased without marked interference with the processes of oxidation in the tissues, (3) to study the influence of oxygen administration in cases of interference with the pulmonary ventilation.

The distribution of oxygen in the arterial and venous blood of normal individuals has been studied by Harrop(1), who found that the average oxygen saturation of arterial blood in 15 normal subjects varied between 100 p c and 94.3 p c with an average reading of 95.5 p c and the average oxygen consumption between 2.6 and 8.3 volumes p c. Stadie(2) carried out similar investigations both in normal persons and in patients suffering from pneumonia. His results show in normals a mean arterial oxygen unsaturation of 5 p c and a venous unsaturation of 26.8 p c. In pneumonia patients the arterial oxygen unsaturation was found in one case to be as low as 68.2 p c all cases in whom there was an arterial unsaturation of more than 20 p c proved fatal. The venous blood in these patients had an unsaturation varying from 14.4 p c to 85.5 p c. The term "oxygen unsaturation" was introduced by Lundsgaard(3), who defined it as the difference between the oxygen content of the blood obtained from the subject and the total oxygen capacity of the same blood when fully saturated with oxygen. Stadie(2) maintains that the arterial and venous oxygen contents run parallel so that the difference between the two is almost constant. This difference between the oxygen contents of arterial and venous bloods we may call the "head of oxygen." Doig(4), in a study of the influence of acute anoxic anoxæmia, also came to the conclusion that the head of oxygen was fairly constant.

I. *The oxygen content of the blood in varying degrees of anoxæmia.*

In the first place it was necessary to investigate the influence of anoxæmia on the supply of oxygen to the tissues and to determine whether there were any conditions in addition to the primary cause of the anoxæmia that modified this supply.

Head of oxygen in asphyxia. The experiments were performed on cats under a constant degree of ether anæsthesia. With the animal in the dorsal position the trachea was opened and a cannula inserted connected with tubing to the ether bottle. The left carotid artery and external jugular vein were exposed. To ensure synchronicity in obtaining specimens of arterial and venous blood there was fixed into the artery a cannula from which the arterial blood was collected by one operator, while the other simultaneously performed venous puncture. The blood was withdrawn into all-glass 5 c.c. syringes. A small amount of liquid paraffin was used as a lubricant in order the more perfectly to exclude air. Each syringe contained a pinch of neutral potassium oxalate to prevent clotting of the blood.

Asphyxia of varying degrees was then induced. To prevent accumulation of carbon dioxide, the animal was made to breathe through a closed circuit in which was placed a jar of soda-lime. In this way a gradual reduction of the oxygen content of the inspired air was induced without any disturbing factors. The oxygen content of the blood was determined by Barcroft's differential method. The results are given in Table I. The results in all the tables are given to the nearest whole number.

TABLE I.

		Percentage saturation of the blood					
		Before asphyxia			During asphyxia		
Exp.	Degree of asphyxia	Arterial	Venous	Diff.	Arterial	Venous	Diff.
1	+	92	68	25	70	47	23
2	++	95	50	45	72	28	44
3	+++	95	55	40	47	18	29
4	+++	95	62	33	17	22	- 5
5	+++	95	54	41	19	21	- 3

Up to a certain degree of asphyxia the amount of oxygen in the arterial blood exceeds that in the venous. The head of oxygen, however, varies within wide limits. When the asphyxia becomes more intense the percentage oxygen saturation in the arteries begins to fall much more rapidly than in the veins and in the extreme stages of asphyxia the

venous blood actually contains a higher percentage of oxygen than does the arterial. These results indicate that the arterial and venous oxygen contents do not run parallel as Stadie has maintained; in other words, the head of oxygen is not a constant, but depends on the degree of pulmonary ventilation amongst other factors. As regards the lower arterial oxygen saturation in the extreme degrees of asphyxia, this is probably accounted for by the oxygen usage of the pulmonary tissue as the experiments of Bohr and Henriques(5) indicate that some oxidation occurs in the lungs themselves.

Effect of nature of tissues drained by the vein on the unsaturation of the venous blood. Krogh and Lindhard(6) have shown that the coefficient of utilisation of oxygen varies with the amount of work done. It is also well known that an organ during activity requires much more oxygen than it does during rest. Accordingly one would expect that there would be a much greater oxygen unsaturation of the blood coming from a muscular part of the body than of that passing from a region with less active tissue. In order to determine the effect of position in the venous system on the oxygen content of the venous blood, simultaneous puncture of the right ventricle and the left jugular vein was performed. This was done with the thorax opened and respiration maintained by a motor pump.

TABLE II.

Exp.	Percentage O ₂ saturation of blood	
	Jugular vein	Right ventricle
1	62	52
2	62	57
3	78	69

Thus the blood from the right ventricle is, as would be expected, always more unsaturated than that from the jugular vein. It is evident, however, that the blood from the jugular vein may be taken as giving a relative measure of the desaturation taking place in the tissues generally.

Effect of asphyxia on the oxygen saturation of the ventricular blood. The thorax was opened and the heart exposed, artificial respiration being maintained, and as the table shows the ventilation was not so good as in natural breathing. Blood was withdrawn from the right and left ventricles by simultaneous oblique punctures: by this method no after-hæmorrhage was caused. The artificial respiration was then stopped for varying lengths of time in the various experiments and blood again withdrawn.

TABLE III.
Percentage O₂ saturation of blood

Exp.	Degree of asphyxia	Before asphyxia			During asphyxia		
		L. vent.	R. vent.	Diff.	L. vent.	R. vent.	Diff.
1	+	80	44	36	51	42	9
2	+	57	28	29	40	28	12
3	++	69	19	50	36	31	6
4	+++	80	40	40	24	13	11
5	+++	74	50	24	15	12	3
6	+++	65	36	29	14	17	3

These results closely resemble those in Table I. The head of oxygen does not remain constant, but varies within fairly wide limits. When the saturation of the blood in the left ventricle falls to below 20 p.c. the head of oxygen becomes very small or may even be a negative quantity, probably as previously indicated, a result of the consumption of oxygen by the pulmonary tissue.

Effect of anæmia on the head of oxygen. In all experiments the second or "anoxæmic" blood was withdrawn after the volume of blood had been depleted. It was therefore necessary to determine the effect of this anæmia. Finkler(7) found that there was a diminished oxygen content of the blood after anæmia had been induced in dogs by bleeding. Schlomowitz, Ronzoni and Schlomowitz(8) produced repeated slight hæmorrhages in dogs, but observed no marked decrease in oxygen consumption until the blood-loss reached 15 p.c. to 25 p.c. of the original volume. Lundsgaard(9) found in patients suffering from anæmia that the oxygen saturation of the venous blood was independent of the total oxygen capacity and that the tissues were able to extract from the blood practically all the oxygen reserve.

The oxygen saturation of the arterial and venous blood was determined before and after a measured quantity of blood was withdrawn.

TABLE IV.
Percentage O₂ saturation of blood

Exp.	Weight of animal in kg.	c.c. of blood withdrawn	Before bleeding			After bleeding		
			Artery	Vein	Diff.	Artery	Vein	Diff.
1. Dog	5.8	63	94	60	34	91	68	23
2. Cat	2.4	25	92	66	26	92	57	35
3. "	2.2	21	94	71	23	95	69	26
4. "	3.2	50	94	61	33	74	59	15
5. "	2.8	50	96	65	31	69	60	9
6. "	2.1	50	92	60	32	51	37	14

After small hæmorrhages there is little change in the oxygen saturation of either arterial or venous blood. When the volume of blood suffers

a more marked depletion there is a marked fall in the saturation of both arterial and venous bloods, as well as a decrease in the head of oxygen.

Effect of temperature on the oxygen content of the venous blood. Hewlett(10), by a series of observations on normal men, showed that changes in room temperature produced marked effects upon the rate of blood-flow in the arm, warmth causing an increase, cold a decrease. The next series of experiments were performed to determine the effect of variations in temperature on the venous oxygen content. Cats, anæsthetised by ether, were used and the femoral vein on each side dissected out. One leg was wrapped up in hot cloths while the other was covered with ice. After ten minutes blood was withdrawn simultaneously from the two veins. The arterial saturation was estimated in blood drawn from the left carotid artery.

TABLE V.

Exp.	Arterial (normal temp.)	Percentage O ₂ saturation of blood			
		Hot		Cold	
		Ven.	Diff.	Ven.	Diff.
1	93	59	34	38	55
2	95	65	30	46	49
3	94	66	28	49	45
4 partly	72	44	28	32	40
5 asphyxiated	70	50	20	41	29

The venous blood coming from the warm limb was 20 p.e. more saturated with oxygen than that from the cold part. When slight asphyxia was induced previous to the withdrawal of the blood, the difference in the oxygen saturation was not so marked. This is probably due to the slowing of the blood flow caused by the asphyxia being more marked in the warm than in the cold limb.

Effect of pneumococcal infection on the total oxygen capacity of the blood. Stadie(2) mentions change in the hæmoglobin as a possible factor in the production of the cyanotic appearance of the pneumonic patient. Peabody(11) induced a profound pneumococcal septicæmia in rabbits and found a rapid and marked fall in the total oxygen capacity of the blood. Peabody(12) also found in a few severe cases of pneumonia a decrease in the total oxygen capacity of the blood. Stadie(13), however, in a series of 16 cases, all of which proved fatal, failed to find any marked decrease.

The following estimations were done to determine whether there was any diminution in oxygen carrying power of the blood of patients

suffering with lobar pneumonia, as compared with the blood of a normal subject (N. M.).

TABLE VI.

Patient	O ₂ capacity of blood as compared with control	O ₂ saturation of venous blood
	%	%
A. P.	96	47
B. H.	104	21
G. C.	99	31
D. W.	103	35

In the cases investigated there was no decrease in the total oxygen-capacity of the blood, although all the patients were suffering from a severe degree of lobar pneumonia. Changes in the hæmoglobin do not therefore account for the high percentage unsaturation of the blood in pneumonia conditions.

The results of the various experiments indicate that the head of oxygen depends upon the following factors. These are much the same as those already mentioned by Lundsgaard.

(1) Degree of arterial saturation as demonstrated by the results of the asphyxia experiments.

(2) Nature and condition of the tissues drained by the vein from which the venous blood is taken. This is evidenced by the different oxygen-saturations of blood from the jugular vein and that from the right ventricle, as also by the effect of variations of temperature on the venous oxygen saturation.

(3) Variations in the amount of available hæmoglobin as is shown by the effect of hæmorrhage.

It will be seen, however, that even when these conditions are within normal limits the difference between the oxygen content of arterial and venous blood varies between a fifth and a half of the total oxygen capacity of the blood. This normal variation is possibly a result of the differences in different animals of the velocity of the blood stream and of the rate of metabolism. A further disturbing factor is the effect of the anæsthetic. Although each animal was kept under a constant degree of anæsthesia, the depth of anæsthesia was no doubt not the same in all the cases. The head of oxygen is of real value as a measure of oxygen consumption only for different conditions in the same animal, but cannot be used for comparison in different animals. Thus it not infrequently occurred that the head of oxygen in one experiment during fairly deep asphyxia was greater than in another where there was no interference with the pulmonary ventilation.

II. *Effect of interference with the pulmonary mechanism on the head of oxygen.*

In acute pulmonary conditions met with clinically, it is found that a greater or less area of the lung tissue is temporarily out of action. In order to determine whether mechanical interference with the pulmonary mechanism was of itself sufficient to produce an anoxæmia in the tissues the following series of experiments were performed.

Effect of pneumothorax on the supply of oxygen to the tissues. Cats were anæsthetised with ether; the carotid artery and jugular vein were exposed and blood withdrawn for "normal" analysis and the thoracic wall on the right side was opened. Digital examination demonstrated the collapse of the right lung, and ten minutes later blood was withdrawn from the artery and vein.

TABLE VII.

Exp.	Percentage O ₂ saturation of blood					
	Before pneumothorax			After pneumothorax		
	Arterial	Venous	Diff.	Arterial	Venous	Diff.
1	93	46	49	71	22	49
2	85	71	14	64	43	21
3	91	47	44	75	17	58

These results show that collapse of one lung produced a marked fall in the oxygen content of the arterial and venous bloods, the reduction being permanent. There is usually an increase in the head of oxygen. It will be seen that the figures for the arterial unsaturation resemble those found by Meakins(14) in cases of acute lobar pneumonia.

Effect of occlusion of one bronchus. Loewy and von Schlotter(15) occluded the main bronchus of one lung without altering the rate of the respiration or circulation, and found that the oxygen content of the venous blood was slightly lowered. The following experiments were carried out to determine the effect of such a procedure on the head of oxygen. Dogs were used in this series. After anæsthesia had been induced, a large plug of cotton wool soaked in oil was inserted into the trachea, and by means of a blunt metal probe was pushed into the left bronchus. The left lung was auscultated to determine whether there was any air-entry into the lung, and if there was, the plug was removed by means of an attached thread and readjusted until no respiratory murmur could be heard. The movement of the left side of the chest was markedly decreased compared with that of the right.

TABLE VIII.

Exp.	Percentage O ₂ saturation of blood					
	Before occlus. of bronchus			During occlus. of bronchus		
	Arterial	Venous	Diff.	Arterial	Venous	Diff.
1	91	74	17	50	18	32
2*	93	75	18	27	21	6
3	95	55	40	72	25	47

* In Exp. 2 it was found on post-mortem examination that the right bronchus was also partially excluded.

These results indicate that occlusion of one bronchus produced a marked fall in the oxygen saturation of the blood, but a slight increase in the head of oxygen. This would indicate either that there is still some circulation in the left lung, or that the flow of blood through the functioning pulmonary tissue was too rapid to allow of complete saturation of the blood.

Effect of histamine. Dale(16) has shown that the main action of this substance is on the bronchial musculature and pulmonary arteries. Anderes and Cloetta(17) studied the effect of histamine on cats, and found a marked rise in pulmonary arterial pressure and a fall in lung volume together with a decrease in oxygen absorption. Accordingly it acts on the whole pulmonary mechanism decreasing its efficiency. In order to determine the effect of histamine on the head of oxygen 0.5 mg. of the drug dissolved in 1 c.c. of saline was injected into the jugular vein.

TABLE IX.

Exp.	Percentage O ₂ content of blood					
	Before histamine			After histamine		
	Arterial	Venous	Diff.	Arterial	Venous	Diff.
1 	94	59	35	78	63	15
2 	87	51	36	72	50	22
3 	94	60	34	81	57	24
3 after 2nd dose	"	"	"	65	50	15
4 1 mg. inj. ...	93	61	32	37	30	7

Histamine causes a diminished oxygen saturation of the arterial and venous bloods together with a fall in the head of oxygen. Whenever the respiratory exchange in both lungs is affected there always occurs a fall in the head of oxygen, although accompanied by a greater desaturation of the arterial blood. This contrasts with what takes place when there is interference with the ventilation of but one lung. In this latter condition there is a rise in the head of oxygen although it too is accompanied by an increased desaturation of the arterial blood.

These experiments vary in two particulars (1) extent of interference with the respiratory exchange, and (2) extent of interference with the pulmonary circulation. The extent to which the respiratory exchange is disturbed may be gauged by the desaturation of the arterial blood, which may be greater when there is unilateral interference with the pulmonary mechanism than when both lungs are involved in the lesser degrees of asphyxia. Yet the head of oxygen is always diminished in the latter condition while it is raised in the former. It would seem, therefore, that the head of oxygen depends on the extent of interference with the pulmonary circulation. Underhill⁽¹⁸⁾ has shown that ligation of one bronchus produced an increase in the pulmonary arterial pressure, and it may be supposed that increased interference with the pulmonary mechanism would lead to a still further rise in pressure with consequent congestion in the right heart. This congestion in the venous system probably leads to an increased percentage in red corpuscles in the blood owing to a concentration of the blood. Thus the venous blood analysed contains a larger number of oxygen carriers per unit volume than does the arterial, and consequently, other things being equal, would give a higher oxygen content. This might also explain in part the higher venous oxygen saturation in extreme degrees of asphyxia.

When the circulation of one lung is intact, there is an increase in the arterial desaturation indicating defective respiratory exchange, but there is no evidence of any back pressure, as after ligation of one bronchus the rise of pulmonary arterial pressure is only temporary, soon regaining its normal level. Accordingly the tissues obtain fully their normal supply of oxygen as indicated by the slight increase in the head of oxygen. It may be that the increase is due to a certain amount of blood being imprisoned in the affected lung, necessitating a greater demand on that which is actively circulating. Underhill⁽¹⁸⁾ has shown that following constriction of the left pulmonary artery with the chest closed, the left lung is as full of blood as the right.

III *Effect of the administration of oxygen*

The following series of experiments were performed to determine the effect of oxygen administration on the oxygen content of the blood in cases of mechanical interference with the vital capacity of the lungs. In series I unilateral pneumothorax was induced in the way previously described. Oxygen was then administered for a period of five minutes by allowing it to pass down the tracheal tube under very low pressure. In series II the left bronchus was plugged and oxygen administered for

a period of five minutes. In series III oxygen was supplied for five minutes previous to the production of unilateral pneumothorax, and thereafter for ten minutes.

TABLE X
Percentage O₂ saturation of blood

Exp.		Normal			O ₂ during pneumothorax		
		Arterial	Venous	Diff.	Arterial	Venous	Diff.
I.	1	95	70	25	92	22	70
	2	93	61	32	91	63	28
II.		Normal			O ₂ during occlusion of bronchus		
	3	95	55	40	97	25	72
	4	93	67	26	90	50	40
	5	94	61	33	93	62	31
III.		Normal			O ₂ previous to and during pneumothorax		
	6	74	48	26	98	57	41
	7	89	41	48	89	45	44
	8	89	78	11	96	—	—
	9	91	77	14	93	43	50

Administration of oxygen produced a rapid return of the arterial saturation to normal and a reduction of the venous unsaturation. When oxygen is administered previous to the production of pneumothorax a fall in the oxygen saturation of the arterial blood is prevented. These results show that an increase of oxygen in the inspired air tends to minimise the effect of any diminution in the vital capacity of the lungs. The effect of oxygen administration was always to maintain the arterial saturation practically to the normal level. The head of oxygen was usually increased but not invariably so. Possibly the rise may be accounted for by the interference with the circulation of one lung, whereas in those cases that showed no change or a fall in the head of oxygen, a decrease in viscosity or some other effect of the increased supply of oxygen, may have more than neutralised the interference to the pulmonary circulation.

SUMMARY.

I. 1. The difference between the percentage oxygen saturation of arterial and venous blood is not constant even in the same animal, and when the saturation of the arterial blood reaches about 20 p.c. the head of oxygen becomes very small and may even be a negative one. It is suggested that this may be explained by the oxygen consumption of the pulmonary tissue.

2. The saturation of the venous blood depends not only on the

saturation of the arterial blood, but upon the nature and condition of the tissues drained by the vein

3 Pneumococcal infection, whilst it may produce a lowering of the percentage saturation of the blood, does not cause a fall in its total oxygen capacity

II 1 Unilateral interference with the pulmonary mechanism such as induction of unilateral pneumothorax or occlusion of one bronchus produces a fall in the oxygen saturation of the blood both arterial and venous, but a small increase in the head of oxygen

2 Interference with the respiratory exchange in both lungs as produced by injection of histamine, partial blocking of both bronchi and induction of asphyxia leads to a decrease in the arterial oxygen saturation and a fall in the head of oxygen

III 1 Administration of oxygen subsequent to interference with the pulmonary mechanism raises the oxygen saturation of the arterial blood to normal level

2 Oxygen administration antecedent to the induction of pneumothorax and continued thereafter prevents any fall in the oxygen saturation of the arterial blood

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THE HEAT-PRODUCTION AND THE MECHANISM OF
THE VERATRINE CONTRACTION. BY W. HARTREE¹
AND A. V. HILL.

(From the Physiological Laboratories of Cambridge and Manchester.)

THE remarkable action of veratrine on skeletal muscle has been the subject of many investigations, but its mechanism remains obscure. The following pages show that, whatever that mechanism, the liberation of energy in the veratrine contraction bears, qualitatively and quantitatively, the same relation to the mechanical response as it does in an ordinary prolonged contraction produced by a succession of shocks.

It was shown by Fick and Böhm⁽¹⁾ in 1872 that the veratrine contraction, in response to a single shock, causes a production of far more heat than the normal twitch. They concluded that the prolonged veratrine contraction is due to a greater intensity of the "chemical processes," and not simply to a slowing of the "restitution processes." They showed also that a short tetanus leads to a greater production of heat than a single shock, as in ordinary muscle. They approached the question of whether the prolonged veratrine contraction is of a tetanic nature, by laying the nerve of a second muscle-nerve preparation upon an excited veratrinised muscle; the absence of any response in the former led them to conclude that the veratrine contraction is not of an oscillatory character. This question has been the subject of many later investigations. Hoffmann⁽²⁾ found that muscles subjected to weak doses of veratrine show an electric response of a pronounced oscillatory character, as does a tetanised, or a voluntarily innervated, muscle: while muscles treated with stronger doses show a smooth, non-oscillatory electric response, as well as a continuous mechanical one. In our investigation we have employed only solutions which, in Hoffmann's nomenclature, would be "strong."

In an ordinary prolonged isometric contraction, induced by a rapid succession of shocks, we have shown^(3, p. 144) that, after a very short interval during the development of the contraction, the *rate* of heat-production (called here for simplicity the "heat-rate") is proportional

¹ Working for the Medical Research Council.

to the force maintained. Expressing the heat-production in work units (grm. cm.), in order to make it directly comparable with the product Tl , T being in grms. wt. the force maintained, and l in cm. the resting length of the muscle, we showed (3, p. 147) that, at 15°C ., in the sartorius muscle of the frog (*Rana temp.*), the ratio (heat-rate)/ Tl , rapidly attains the constant value 0.61. We argued that a prolonged stimulus causes a steady production of lactic acid, which is "removed" at a rate depending upon its concentration, the mechanical response being due to the momentary presence of the free acid in the neighbourhood of certain sensitive structures in the muscle: in this way the force maintained, once a steady state is reached, will be proportional to the rate at which the lactic acid is being formed and removed, i.e. to the heat-rate.

It was possible that veratrine might act at one or other of several different points in the mechanism: it might for example delay the "removal" of the acid either (a) by slowing the normal chemical processes of relaxation, or (b) by rendering the sensitive structures of the muscle abnormally receptive to lactic acid, and so much slower in parting with it: or, on the other hand, it might cause the liberation of acid, once started by a shock, to proceed unchecked by the normal "escapement" which limits the liberation of acid and energy in a twitch. Now anything which acts upon the rate of "removal" of the acid must affect the ratio (heat-rate)/ Tl : the "efficiency" with which a prolonged contraction is maintained, which is the inverse of this ratio, is increased by anything which slows relaxation, e.g. by a fall of temperature or by fatigue (3, p. 148). Hence, if by any means veratrine were to slow the normal processes of relaxation, the ratio (heat-rate)/ Tl should be far smaller than normally: the maintenance of the contraction should be far more efficient. If, on the other hand, the action of veratrine be, either to produce a tetanus or to abolish, in whole or in part, the normal check to an unlimited and continual breakdown in response to a shock, leaving the physico-chemical nature of the mechanical response to that breakdown, and of relaxation, quite unaltered, then we should expect to find, during the prolonged isometric contraction of a veratrinised muscle, (a) a prolonged production of heat as Fick and Böhm found, (b) that the curves of heat-rate and of tension should run parallel, and (c) that the ratio, (heat-rate)/ Tl , should have the same absolute value as in a prolonged contraction produced at the same temperature by a succession of shocks. All three of these expectations have been verified.

Method. A pair of sartorius muscles of *Rana temp.* was mounted on a thermopile, and subjected to a small initial tension, and the course

to the force maintained. Expressing the heat-production in work units (grm. cm.), in order to make it directly comparable with the product Tl , T being in grms. wt. the force maintained, and l in cm. the resting length of the muscle, we showed (3, p. 147) that, at 15° C., in the sartorius muscle of the frog (*Rana temp.*), the ratio (heat-rate)/ Tl , rapidly attains the constant value 0.61. We argued that a prolonged stimulus causes a steady production of lactic acid, which is "removed" at a rate depending upon its concentration, the mechanical response being due to the momentary presence of the free acid in the neighbourhood of certain sensitive structures in the muscle: in this way the force maintained, once a steady state is reached, will be proportional to the rate at which the lactic acid is being formed and removed, i.e. to the heat-rate.

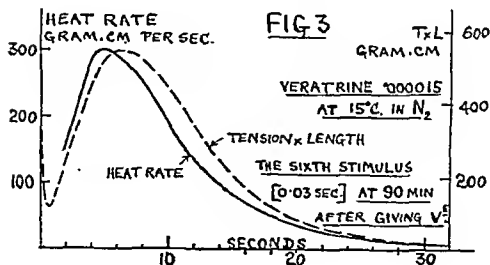
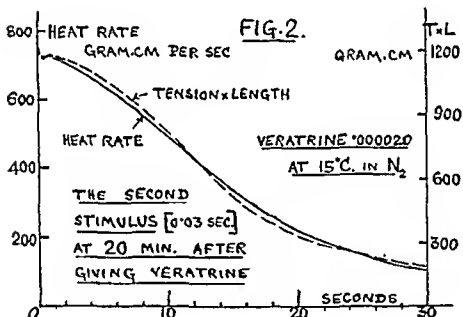
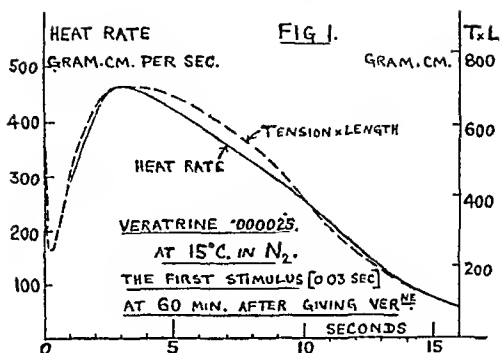
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Method. A pair of sartorius muscles of *Rana temp.* was mounted on a thermopile, and subjected to a small initial tension, and the course

of the heat-production after a stimulus found by analysing the photographic curves of galvanometer deflection, as described previously (4, p. 102). Most of the experiments were made at 15°C ., the muscle being in nitrogen which had been freed from oxygen by passing it through two bottles of alkaline pyrogallol: in this way, heat-production due to oxidative recovery is entirely eliminated. A suitable interval for the analysis was found to be 1 sec. during the first 20 secs. of the contraction. After that a 5 sec. interval could be used without loss of accuracy. The tension was recorded on a smoked drum by a tension lever, the contractions being practically isometric.

After a few preliminary stimuli, to determine, in the normal muscle, (a) the strength of the maximal stimulus (always a short tetanus of 0.03 sec.), and (b) the corresponding heat-rate and tension curves, the veratrine solution was introduced into the chamber, and left in for four or five minutes, after which it was blown out by nitrogen. The strength of the veratrine, made up in Ringer's solution, was usually .002 p.c. or less: with stronger solutions the galvanometer zero was very unsteady for an hour or more, and in this case, as is well known, see (5), the first tension curves made show a sharp fall immediately after the initial quick response followed by a subsequent rise, little, if any, greater than that due to a very short tetanus. With weaker solutions the galvanometer zero was steady and records could be taken about a quarter of an hour after the veratrine solution had been removed. In order to record the relatively enormous heat-production of the veratrine contraction, the deflection was reduced by the introduction of a resistance about 15 times that of the thermopile and galvanometer together, the sensitivity of the galvanometer itself being left unchanged. Control curves were made on the dead muscle and the analysis conducted as usual.

Results. It is known that, with relatively weak solutions of veratrine, the contraction is very similar, in height and form, to a tetanus obtained by discontinuous stimulation (Fig. 2): whereas, with stronger solutions, a sharp twitch is found preceding the prolonged contraction (Fig. 1). With weaker solutions, the first one or two contractions are of the simple prolonged form; in later contractions, however, the initial twitch, followed by the later prolonged rise, is pronounced (see Fig. 3, which shows a sixth contraction). The unsteadiness of the galvanometer, together with the spontaneous fibrillation which Prof. Langley informs us is often visible, in a muscle treated with a strong solution of veratrine, may be regarded as an exaggeration of the weakening of normal control of breakdown caused by weaker solutions. Such spontaneous breakdown



would have the same effect as artificially induced activity, in changing the character of the response: consequently the first contraction of a muscle subjected to a strong solution is like the later one of a muscle treated with a weaker.

On comparing the heat-rate with the tension at any moment, it is found that, throughout the contraction, and certainly as a first approximation, the two are definitely proportional. See Figs. 1 to 3, in which the heat-rate and the product Tl are plotted throughout in the same units of energy (grm. cms.), to such scales that their maxima coincide: in this way the close agreement of the two curves is convincing evidence that, in a veratrinised muscle, the prolonged contraction is accompanied by a proportional prolonged heat-production.

When, as in Fig. 3, the agreement between the curves of heat-rate and of Tl is not quite exact, it is found that the fall of the latter curve tends to lag behind that of the former: this divergence is more pronounced when the muscle has been fatigued by earlier contractions. We have shown elsewhere (3, p. 148), that any factor, such as fatigue, which tends to slow the processes of relaxation, results in a diminution of the ratio, (heat-rate)/ Tl , and if the slowing effect of fatigue on the "removal" processes be considered to occur during the single veratrine contraction, it is natural that the tension curve should fall less rapidly than that of heat-rate. Any want of absolute agreement between the curves of heat-rate and of tension, is probably due to this effect of oncoming fatigue.

In the figures, the quantities plotted are expressed per grm. of muscle.

As regards the absolute value of the ratio, (heat-rate)/ Tl , its average during relaxation in the *third* contraction in five different experiments at 15° C., was as follows:

0.60, 0.53, 0.54, 0.61, 0.60; Mean, 0.58;

the unit being sec⁻¹.

For the first stimulus (which was not always well observed, owing to the unknown magnitude of the expected galvanometer deflection) the corresponding number was about 0.63, whereas for the fifth stimulus (in which the tension was sometimes too small to be well observed) it was about 0.53. Here again the effect of fatigue is to diminish the ratio. The higher values compare very exactly with those found previously by ourselves at 15° C. (3, p. 147) for the case of a prolonged tetanic contraction, the mean deduced from a number of observations made by another method being about 0.61. There can be no doubt therefore that the efficiency with which the prolonged contraction is maintained is almost exactly the same in the veratrine contraction as in the normal tetanus.

As a verification of the method one set of experiments was carried out in oxygen at 15° C., giving a long tetanus either of diminishing or of

constant strength, to a muscle untreated with veratrine, and completing the analysis as in the veratrine experiments. In this case also it appeared that the heat-rate throughout is very nearly proportional to Tl , and that about the same absolute value of the ratio is obtained.

We see therefore that the action of veratrine is limited to its effect upon the extent and duration of the liberation of energy, and presumably therefore of lactic acid, following a stimulus. In the normal twitch at 15°C . the liberation of energy is exceedingly rapid: even at 10°C ., as we have shown (4, p. 112), it appears to be complete within 0.2 sec., although in a twitch some of the energy is stored in the potential mechanical form, and reappears after a short interval during relaxation. There is clearly some passage of escape, for the energy and lactic acid, which is opened by a shock and which, in normal muscle, rapidly closes again. This "passage" may not be of a mechanical or a structural nature, though we have supposed (3, p. 140), for the sake of definiteness, that it is opened and closed by a momentary change in the permeability of some membrane within the fibre. The "passage" can remain open, in a normal muscle, only in consequence of a rapid succession of shocks. In the muscle subjected to a large dose of veratrine the instability of the galvanometer zero shows that the "passage" is so nearly open that small spontaneous outbursts of energy may occur. In the muscle subjected to a weaker dose, the "passage," once opened by a shock, appears to remain open for some time, allowing considerable quantities of lactic acid to pour out, with a prolonged development of tension and heat. In very weak doses the oscillatory nature of the electric response (Hoffmann) suggests that the passage is opened discontinuously by something of the nature of a tetanus. According to Lamm (6), the action of veratrine resides in a change which it produces in the permeability of some membrane, since the effect of veratrine may be largely antagonised by that of calcium salts. Such evidence of course is only circumstantial, and the description of the regulating mechanism itself as a membrane is hypothetical, though possibly useful as giving definition to the undoubted fact that some regulating mechanism certainly exists, cutting short and controlling the liberation of energy following a single shock. Our experiments show that the effect of veratrine is to be ascribed to its action on this regulating mechanism, causing the liberation of energy, for some reason or another, to be much more extensive and prolonged.

SUMMARY.

In the prolonged isometric contraction of a veratrinised muscle there is a prolonged evolution of heat, the rate of heat-production being proportional throughout to the force maintained. The absolute value of the ratio, $(\text{heat-rate})/(\text{force} \times \text{length})$, is the same as that found in the ordinary prolonged contraction set up by a tetanic stimulus. These facts show that the effect of veratrine cannot be ascribed to a slowing of relaxation, or of the chemical processes by which the acid which excites the mechanical response is removed from the site of its activity; it can only be supposed that it puts out of action, more or less completely, the regulating mechanism by which the duration and extent of the liberation of energy (and lactic acid) following a single shock, are limited and controlled.

The expenses of this research have been met, in large part, by a grant from the Royal Society.

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THE ALL-OR-NOTHING RESPONSE OF SENSORY NERVE FIBRES. BY E. D. ADRIAN AND ALEXANDER FORBES.

(From the Physiological Laboratories of Cambridge and of the Harvard Medical School.)

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I. THE RELATIONS BETWEEN STIMULUS AND IMPULSE IN SENSORY NERVE FIBRES

In the motor nerve fibres of the frog's sciatic the disturbance which constitutes the impulse appears to obey the all-or-nothing rule. The size or intensity of the disturbance at any point does not depend on the strength of the stimulus which has given rise to it, or on the state of the fibre through which it has passed, but only on the local conditions at the point at which it is measured. If these remain constant, the intensity of the disturbance is invariable provided that it occurs at all, and any gradation in the response of the effector organ is brought about by gradation in the number of nerve fibres in action or in the frequency of the impulses in them. The proof of this statement is derived from several converging lines of evidence(1), but hitherto this evidence has related almost exclusively to the motor nerve fibres of the frog. The present research was undertaken to find out whether the all-or-nothing relation

holds good for afferent fibres as well as for efferent, and incidentally to investigate the relation in mammalian nerves, since it is in mammalian preparations that the most interesting questions in this connection have arisen.

The extreme range of gradation of certain sensations and certain reflex responses makes it difficult, at first sight, to believe that sensory fibres do not differ from motor fibres in possessing some means of varying their response apart from changes in frequency. It is unlikely that the conduction processes can differ radically in the two kinds of nerve fibre, but although the all-or-nothing reaction is the normal reaction of the motor fibre, yet it is not the only reaction of which the fibre is capable, and under artificial conditions it is easily brought into a state in which the intensity of the impulse is variable. It seemed possible to us that this state of affairs might be the normal condition of the sensory nerve fibre. The state referred to is that in which conduction takes place with a decrement, so that the impulse becomes smaller and smaller, or less and less able to travel, as it passes along; it can be produced in the frog's sciatic by treatment with narcotics, lack of oxygen, deprivation of ions in the perfusing fluid, etc. When the fibre conducts in this way, the size of the impulse at a given point does not depend on local conditions only, since it will be greater or smaller according as the impulse has travelled a short or a long distance in the affected region. This gradual dying out of the impulse seems to occur normally in certain rudimentary types of conducting tissue, *e.g.* the nerve net of the sea anemone⁽²⁾, the pseudopodia of *Diffugia*⁽³⁾, etc. In the vertebrate motor nerve fibre the mechanism of conduction has become more efficient and the impulse is normally conducted without any change in size, although adverse conditions cause a reversion to the less perfect form of reaction. It is therefore quite possible that conduction with a decrement may be a normal event in fibres which are sensory and not motor in function, and if so we need not reckon with the all-or-nothing reaction in explaining the gradation of reflex activity.

But although decremental conduction means that the size of the impulse depends on the distance it has travelled, it does not follow that it depends also on the strength of the stimulus which sets it up, for the setting up of an impulse may involve different processes from those concerned in its propagation from one section to the next. In the primitive nerve network, however, the size of the impulse does appear to vary with the stimulus, and there is evidence that medullated nerve fibres react in the same way when a decrement is produced artificially.

If this evidence can be accepted it is clear that we have a possible explanation of grading in the sensory nerve fibres.

As the experiments bearing on this point did not seem to us altogether conclusive we have tried to determine how far the size of the impulse can be made to vary with that of the stimulus in a fibre which conducts with a decrement, and we have then examined various mammalian nerve trunks and reflex preparations to see how far this will account for the grading in a reflex arc.

Response to stimuli in a region of decrement. It has been shown in the frog's nerve-muscle preparation that when an impulse set up on the proximal side of a narcotised region fails to reach the muscle, it fails for all strengths of stimulus, *i.e.* an increase in the stimulus does not increase the capacity of the impulse to pass through the region of decrement. Thus the size of impulse which can be set up in a normal nerve is independent of the strength of stimulus. But Lodholtz⁽⁴⁾ and Rehorn⁽⁵⁾ have shown that if the stimuli are applied within the decremental region there is a stage of narcosis in which the strength of stimulus required to set up an impulse which will pass to the muscle is greater and greater the longer the distance which the impulse must travel in the region. Since the local excitability must be the same throughout, it appears to follow that the impulse set up by a weak stimulus can only travel a short distance without extinction, whereas that set up by a strong stimulus can travel further. Thus if an impulse is set up in a region of decrement the initial size seems to depend on the strength of the stimulus. Whether this conclusion is valid or not depends on whether the stronger stimuli are accurately localised. A strong stimulus might succeed where a weak one fails, because it could spread down the nerve and start the impulse from a point nearer the muscle. As the evidence on this point did not seem to be conclusive, we have repeated the experiment, with certain modifications, taking special precautions against current spread.

The frog's sciatic gastrocnemius preparation was set up in a chamber shown diagrammatically in Fig. 1, so that the nerve passed through three chambers 9.5 mm. in diameter, through which an alcohol solution could be passed. The nerve could be stimulated at the slots *A* or *B* by sending a current through the fluid from one chamber to the next; in the later experiments a third partition at *A'* gave a third point of stimulation. With electrodes of this form the danger of current spread is very slight. The stimuli used were single break shocks from a coreless induction coil. The primary circuit could be made or broken by the

release of a spring key in which an amalgamated copper point dipped into a pool of mercury, or by the opening of one of the knock-down keys of a Lucas pendulum. The strength of stimulus was controlled by varying the resistance in the primary circuit, the coil distance remaining fixed, and the stimulus is said to be of unit strength when the resistance in the primary is 500 ohms, 10 units when the resistance is 50 ohms, etc.

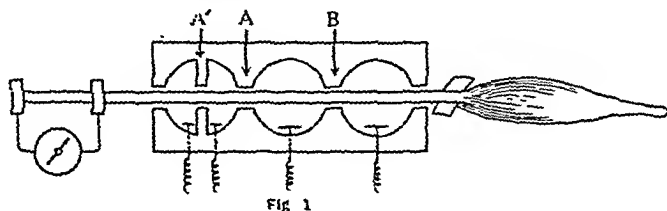


Fig. 1

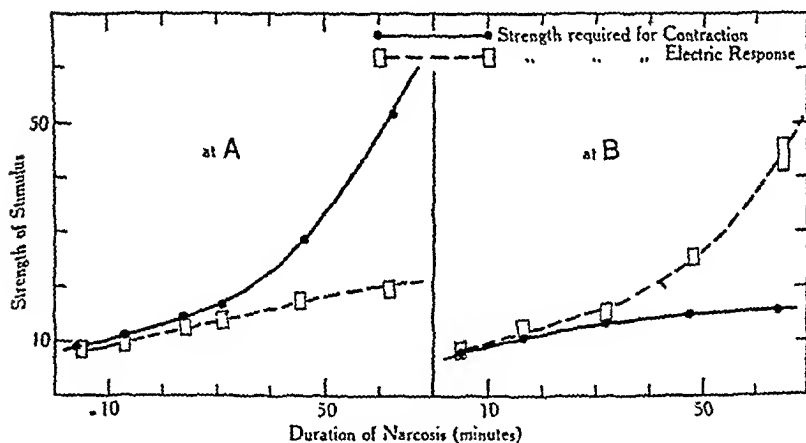


Fig. 2

Fig. 1. Arrangement of narcotising chamber.

Fig. 2. Strength of stimulus required at different stages of narcosis.

The distal end of the nerve was left in connection with the muscle and the arrival of an impulse at this end was shown by the muscular contraction. The proximal end lay on two non-polarisable electrodes connected with a string galvanometer, so that the arrival of the impulse at this end could be detected by a movement of the string. This was sometimes determined by observing its magnified image, but in all significant observations during narcosis photographic records were taken on cinematograph film. As the monophasic response causes a more visible excursion of the string than the diphasic, the nerve was crushed in the neighbourhood of the proximal lead.

When the stimulus is at *A* the impulse will have to pass through

10 mm. of narcotised nerve before reaching the galvanometer leads, and through 20 mm. before reaching the muscle. When the stimulus is at *B* these distances will be reversed. Six experiments on different preparations were made, and each showed a stage during narcosis in which a weak stimulus at *A* caused an electric response but not muscle contraction, and a weak stimulus at *B* caused a contraction but not an electric response, whereas a strong stimulus at *A* or *B* caused both electric response and contraction. Thus a stronger stimulus was required to make the impulse travel 20 mm. than to make it travel 10 mm.; in other words the distance travelled by the impulse varies with the strength of the stimulus. The results of a typical experiment are shown in Fig. 2. It will be seen that after 70 minutes perfusion with alcohol a much stronger stimulus was needed when the impulse had to pass 20 mm. of narcotised nerve and this increase applies to impulses travelling in either direction.

It remains to consider whether the effects of the stronger current are due to its spreading out and setting up an impulse at a distant point. Tests with a nerve damaged by crushing locally showed that a hundred-fold increase of current was needed to make the stimulus spread from one slot to the next. Now in two experiments the nerve was stimulated at *A'* as well as at *A* and *B* (Fig. 1). In one of these a stage was reached at which the strength needed to give a contraction was 81 units at *A'*, and 54 at *A*, whilst that for an electric response was only 2.5 units at either point. If these differences are due to current spread we must suppose that the stimuli from *A'* and *A* both spread down the nerve to the same point (since both are just adequate). But they cannot do this unless the stimulus at *A'* is 100 times as large as that at *A*, whereas in reality it is only 1.5 times as large. The other experiment gave a similar result.

We may conclude that the strong currents are effective, not because they spread down the nerve, but because they set up larger impulses, and there is therefore no doubt that in the late stages of narcosis the size of the impulse varies with the strength of the stimulus which gives rise to it. This conclusion confirms that of Lodholtz and of Rehorn.

The amount of grading in different stages of narcosis. In the preceding section we have only considered the later stages of narcosis where the decrement in unit length is large. On theoretical grounds it might appear that the degree of decrement should make no difference to the relation between stimulus and initial size of impulse, since the slightest decrement implies that the nerve no longer reacts on the all-or-nothing principle.

There is, however, abundant evidence that wide variations in the initial size of the impulse cannot be produced by altering the strength of the stimulus unless the degree of narcosis is considerable. Thus Lucas found that the threshold stimulus in a lightly narcotised area of the frog's sciatic was the same throughout the area (6), although there was no doubt that the nerve was conducting with a decrement. The same point is shown in Fig. 2 where the thresholds rise equally for the first 30 minutes of the narcosis. During this period there was no doubt that the nerve was conducting with a decrement, for it was found that a small impulse set up during the relative refractory phase was able to pass through 10 mm. of the affected nerve but not through 20 mm.

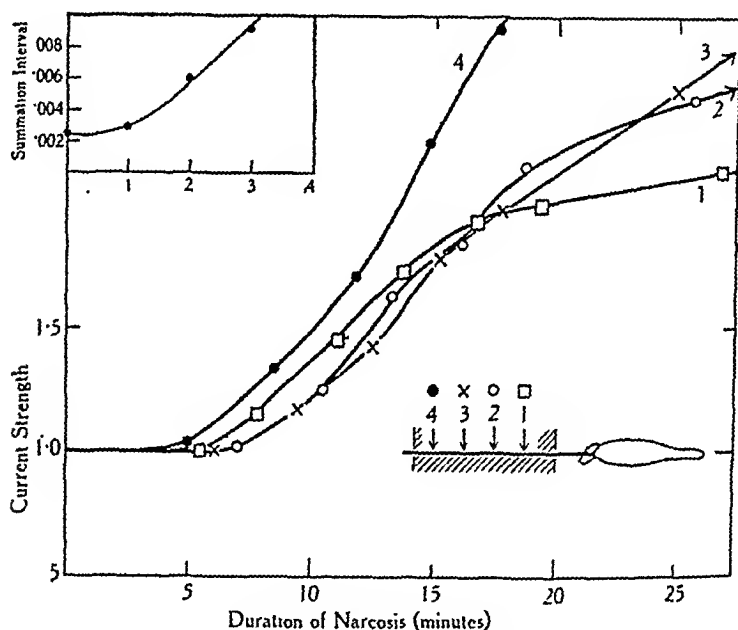


Fig. 3. Rise of threshold during early stages of narcosis.

The progress of events in the earlier stages of narcosis is shown more clearly in Fig. 3, which records an experiment on the frog's sciatic in which the strength required for contraction is measured at four points in a narcotised region. For the first 15 minutes the current strength rises equally at all four points, but later on this state is gradually transformed into one in which the increase in threshold varies with the distance which the impulse must travel in the narcotised area. The small inset in the figure gives the least interval for muscular summation at each electrode 15 minutes after narcosis began. This increases as the distance from the

muscle is increased (cf. Lucas, *loc. cit.*) and there is therefore little doubt of the existence of a decrement in conduction before there is any sign of divergence of the thresholds.

What is the relation between strength of stimulus and initial size of impulse in this early stage of narcosis? Evidently a stimulus of the threshold strength sets up an impulse large enough to travel the maximum distance in the narcotised region without extinction, and a stimulus weaker than this sets up no impulse at all—for if it did, it would be effective at the more distal electrodes. Thus there must be a lower limit to the initial size of the impulse in the early stages of narcosis. Above this lower limit an increase in the stimulus may well cause an increased impulse, although this is merely an inference from what happens at a later stage of narcosis. At a later stage the limiting size becomes smaller and the range of variation correspondingly greater.

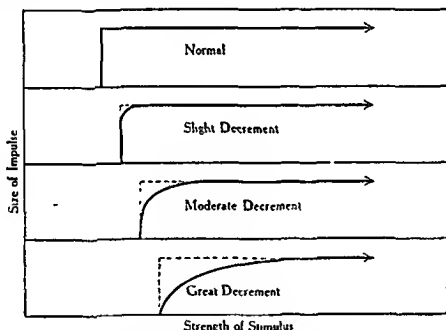


Fig. 4. Probable relation between stimulus and initial size of impulse in different stages of narcosis.

We may therefore represent, by the diagram in Fig. 4, the relation between the strength of stimulus and the initial size of the impulse set up in a region of decrement. This shows that in the normal nerve no variation in the size of the impulse is possible: when there is a slight decrement in conduction through unit length, as in the early stages of narcosis, the threshold stimulus sets up an impulse slightly smaller than the normal and an increase in the stimulus will increase the initial size of the impulse until it reaches an upper limit equal to that in the normal fibre. If the decrement in unit length is greater, the threshold stimulus sets up a smaller impulse and there is a greater possibility of gradation.

The threshold strength in the figure is represented as increasing as the decrement increases, since this would occur if the decrement was produced by narcosis. The form of the sloping part of the curve is entirely conjectural.

The conclusions embodied in this diagram seem to us to be of considerable interest to the theory of nervous conduction, but for the present we are concerned only with their bearing on the question of gradation in sensory nerve fibres. From this point of view the most important deduction is that there can be very little grading in the initial size of the impulse unless the fibre is in such a state that the impulse suffers a considerable decrement in travelling through unit length. It should be a simple matter therefore to discover whether there is normally such a degree of decrement in the sensory fibres as would admit of an appreciable variation in the initial size of the impulse set up by a single stimulus.

Extent of decrement in mammalian sensory fibres. We have used two methods to detect the presence of a decrement in conduction in sensory nerve fibres, and both of these depend on the use of a mammalian nerve removed from the body and set up in a moist chamber. The internal saphenous of the cat is a suitable nerve since it contains no motor fibres and there is no difficulty in obtaining a length of 6-7 cm. from an animal of average size. A certain amount of caution, however, is needed in interpreting the results obtained from surviving mammalian nerves, particularly in regard to decremental conduction. The conditions of survival have been worked out by one of us¹, and it has been found that the nerve is in the most favourable state if it is dissected out with the least possible amount of traction, preserved from contact with damaged tissues and removed from the body not later than a few minutes after the death of the animal. A nerve prepared in this way will often continue to give an electric response for several hours, but in spite of careful dissection some nerves refuse to conduct at all in these conditions, and at best the nerve is in less favourable surroundings than it was in the body. Thus we might often expect to find a decrement in conduction in the surviving nerve though none was present before. On the other hand it is extremely unlikely that the nerve will conduct better out of the body than in it, so that if we find that the isolated nerve conducts without decrement we may be fairly certain that there was none before its removal.

The first method we have used consists in measuring the size of the

¹ A. F. An account of this work will appear shortly in the *American Journal of Physiology*.

electric response called up by an impulse which has travelled (*a*) a short, and (*b*) a long distance from its point of origin. If any reduction is found, we may safely infer that the "propagated disturbance" has been conducted with a decrement. The nerve was set up in a moist chamber at room temperature and connected with stimulating electrodes and non-polarisable leads to the string galvanometer. As the number of fibres

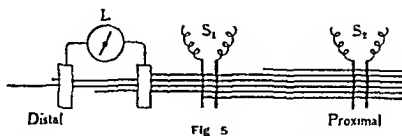


Fig. 5. Arrangement of electrodes on internal sphenous nerve.

in the trunk increases in passing towards the central nervous system, the distal end of the nerve was placed on the galvanometer leads (*L*, Fig. 5) and the stimulating electrodes were arranged nearer the proximal end at S_1 and S_2 . With this arrangement the number of fibres under the galvanometer leads is constant and all of them will be brought into play by a stimulus whether it falls at S_1 or S_2 . The experiment consisted in photographing the monophasic response to stimuli sent in alternately at S_1 and S_2 . The strength of stimulus was so adjusted that a maximal response was given at either point, and the direction of the current was repeatedly reversed so that any artefact due to it could be distinguished from the true electric response. It was found that the threshold stimulus was much the same at either S_1 or S_2 , and that the maximal response was given by a stimulus about three times as strong as the threshold.

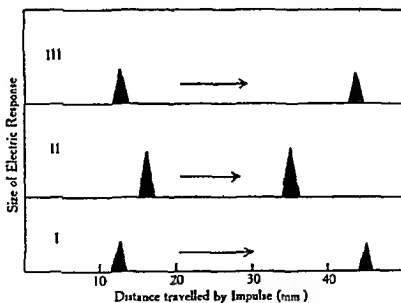


Fig. 6. Size of electric response after transmission through different lengths.

Three experiments were made on the internal saphenous and their results are shown in Fig. 6, which relates the size of the electric response to the distance which the impulse has travelled down the nerve. The size of the response is obtained by measuring the photographic records with a comparator and taking the average value for a series of responses. The figure shows that if there is any decrement at all it must be very small. A survey of all the measurements does appear to show that the response due to a stimulus at S_2 is slightly smaller than that from S_1 , but the difference is within the limits of experimental error. If we may accept the conclusions stated in the last section as applying to mammalian nerve fibre, it is clear that there could be very little grading in the initial size of the impulse unless the decrement in unit length was much greater than that shown in these experiments.

The second, and more delicate method of detecting a decrement in conduction is to produce impulses of very small intensity by stimulating during the period of recovery from a previous impulse and to see how far these can travel without extinction. With the arrangement shown in Fig. 5, if there is any decrement we should find that the small impulse set up in the early stages of recovery at S_2 would fail to reach L , although an impulse of equal size starting at S_1 would be successful. It would follow that the least interval between stimuli required to produce a double electric response at L would be greater when the stimulation is at S_2 than at S_1 , because the second impulse must be initially larger if it starts from S_2 , and it must therefore be set up at a later stage of recovery.

To find the interval necessary for a double electric response the nerve was stimulated alternately at S_1 and S_2 with groups of two stimuli separated by gradually diminishing intervals. The stimuli were delivered by a Lucas pendulum breaking the circuits of two induction coils, and the resulting electric responses were photographed with the string galvanometer. The first series of exposures was developed whilst the experiment was still in progress and an inspection of the film gave a rough idea of the critical interval; the exact value could then be determined by more careful exploration.

Fig. 7 records the intervals measured in three experiments. Those marked I and II are taken from the same nerves as Experiments I and II in Fig. 6. The size of the rectangle shows the limits of accuracy of measurement in each determination. In the lowest line, Exp. I, it will be seen that there is no appreciable difference in the interval necessary at the two points, although the impulses have to travel nearly four times

the distance when they start from the further pair of electrodes. In Exp. II the interval is slightly longer at the greater distance so that in

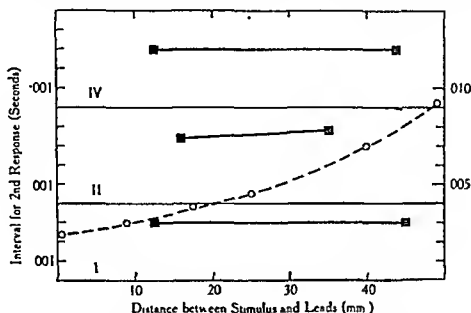


Fig. 7. Intervals required to give a double electric response.

this nerve the impulse probably undergoes a small decrement in conduction. In Exp. IV there is no difference. This experiment differs from the others in that the stimulus was applied to the lower end of the nerve and the response led off from the central end so that the impulses were passing in the direction of their natural flow in the body. The dotted line in Fig. 7 is taken from an entirely different source to show the way in which the interval should change if the decrement in unit length was considerable. It shows the intervals required to give a summated contraction in the frog's gastrocnemius when the stimuli are sent in at different points along a narcotised portion of the sciatic nerve. In this case the interval rises rapidly with increase of distance, although the decrement was not great enough to extinguish a full-sized impulse in 40 mm.

It will be seen that both lines of experiment lead to the same conclusion. In a mammalian sensory nerve the impulse undergoes at most a very slight decrement in conduction through unit length and often none that can be detected at all, and this in spite of the fact that it is exposed to conditions which might well produce decremental conduction. It is indeed surprising that this was not met with more often, seeing that a good many nerves failed to conduct at all when they were set up in the chamber, but it is probable that the decremental state in a medullated fibre is essentially unstable and that the nerve deteriorates rapidly as soon as it has set in. At any rate it is clear that under normal conditions

the sensory nerve fibre agrees with the motor nerve fibre in that the impulse travels down it with little or no reduction in intensity.

The all-or-nothing reaction in sensory fibres. If the sensory fibre of a mammal does not differ fundamentally from the motor fibre of a frog, the conclusion arrived at in the last section means that little or no grading is possible in the initial size of the impulse. The point may be tested directly, however, by a method like that employed for the motor nerve (see p. 303). Stimuli of different strengths are applied to the proximal end of a nerve and the impulses set up are allowed to travel into a narcotised area. If there is any grading in the initial size of impulse there should be a stage in the narcosis in which the impulse set up by a weak stimulus is extinguished in the affected area whilst that set up by a strong stimulus can still pass through. For the sensory nerve the electric response must be used instead of the muscular contraction to show the failure or success of the impulse in passing the decremental region.

The internal saphenous nerve was set up in a moist chamber, the central region, 35 mm. in length, passing through a trough which could be filled with 5-6 p.c. alcohol solution (Fig. 8). The stimulating electrodes were placed on the proximal end of the nerve and the electric responses led off from the peripheral end, this end being crushed to give a monophasic response. The experiment consisted in determining what strength of stimulus was needed to give (a) a minimal, and (b) a maximal electric response at different stages in the narcosis until the failure of conduction was complete. This could only be done by photographing the responses to a series of stimuli of different strengths covering a range wide enough to include the threshold and the maximal strength. The series was repeated at frequent intervals during the narcosis and a complete experiment would involve as many as 150 photographs on a strip of film 30-40 feet long.

The results of two such experiments are shown in Figs. 9 and 10. The strengths of stimulus are expressed on the same scale as in Fig. 2, i.e. a coreless coil is used at a fixed coil distance and the stimulus is said to be of unit strength when the resistance in the primary is 500 ohms, two units when the resistance is 250 ohms, etc. The limits of accuracy in the measurement of the threshold, etc. are shown by the small rectangles: the black triangles rising from the base line mark the height of the maximal electric response at different stages in the narcosis.

The two figures agree very closely with those obtained from the motor nerve fibres of the frog (7). As the electric response becomes smaller, the

strength required for a minimal response remains constant or rises, and that required for a maximal response falls. These changes are readily

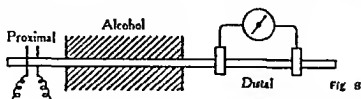


Fig. 8

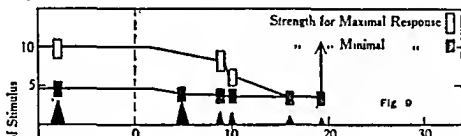


Fig. 9

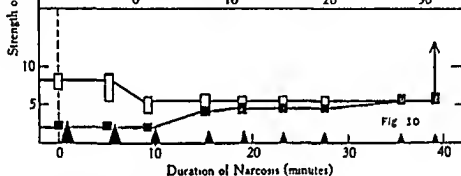


Fig. 10

Fig. 8. Arrangement of electrodes, etc.

Fig. 9. Strength of stimulus needed for maximal and minimal electric response during final stage of narcosis.

Fig. 10. As Fig. 9.

explained by the early failure of some of the fibres, which may happen to be either the most excitable or the least excitable in the nerve. But the important point concerns the strength of stimulus which is effective just before the complete failure of conduction. In Fig. 9 this is no greater than the original threshold value. Thus the stimulus which remains effective until complete failure is one which was originally only just strong enough to produce any effect at all, so that the impulse which it sets up can withstand just as great a decrement as the impulse set up by a stimulus many times as strong. This can only mean that the size of the impulse does not depend at all on the strength of the stimulus. In Fig. 10 the final strength is greater than the original threshold value, but smaller than that originally required to give a maximal response. If we assume an all-or-nothing relation between stimulus and impulse in each fibre the result is very easily explained, for it means no more than the failure of the most excitable and the least excitable fibres before those of intermediate excitability. If we suppose that the size of the

impulse in each fibre varies with the stimulus we might account for the rise in the threshold strength as due to the failure of small impulses set up by weak stimuli, but we have still to explain the fall in the strength required for a maximal response. If this fall is due to the early failure of certain fibres (and no other explanation seems possible) we must admit that the rise in the threshold strength may be due to the same cause. There is therefore no positive evidence in favour of any gradation in the size of the impulse and very strong evidence against it.

Evidence of a slightly different kind is given in Fig. 11, which is plotted from the same experiment as that shown in Fig. 10. Fig. 11 shows

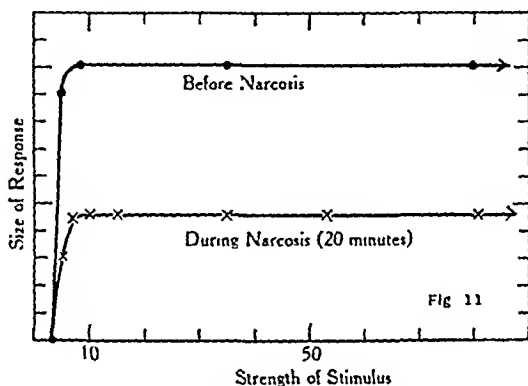


Fig. 11. Strength of stimulus and size of electric response before and during narcosis.

the relation between the size of the electric response and the strength of the stimulus before and during narcosis. It will be seen that the response is considerably reduced by the narcosis and that it cannot be restored to its former value by any increase in the strength of the stimulus.

Results of the type shown in Figs. 10 and 11 were found in all the other nerves which were examined. These were three internal saphenous nerves (two from cats and one from a rabbit) and two sciatic nerves, included to show any possible difference between sensory and mixed nerve trunks. As a rule the narcosis was repeated several times with each nerve, the alcohol being replaced by Ringer's fluid as soon as conduction was suspended. In no case was there evidence of a stage in narcosis in which the impulse was better conducted when the stimulus was increased in strength.

It must be admitted that this evidence does not show that an increase in the strength of the stimulus has no effect whatever on the size of the impulse, but it does show that there can only be a small range of

strengths which cause any variation. In Fig 9 just before the failure of conduction a stimulus of 4.5 units gives an impulse of maximal size, whereas one of 3 units is not strong enough to set up any impulse at all. In Fig 10 the possible range within which variation may occur is greater, since the original threshold is 2 and the final strength 6, although it is highly probable that the threshold strength has increased in the course of narcosis owing to the failure of some of the fibres. In the other experiments on the internal saphenous the limiting strengths were 2.5-4.5, 2-4.5 and 5.5-6.5. There is no evidence that variation does occur between these extremes, but the possibility has not been disproved as the method of experiment does not admit of greater accuracy.

Another possibility which cannot be tested is that the nerve may contain a few fibres which give a graded response but are not numerous enough to have any observable effect on the response of the entire nerve trunk. For the majority of fibres, however, we may conclude that the range of strength of stimulus which may possibly give rise to gradation in the initial size of the impulse is so narrow that such gradation could be of little value in the working of the central nervous system, and it could not possibly account for the wide range of response in the reflex arc.

II GRADATION IN THE REFLEX RESPONSE

Effects of supramaximal stimuli. We come back to the difficulty with which we were faced at the beginning—*i.e.* how to account for graded reflexes without grading of impulses in each fibre. There are, of course, two methods of grading which involve no difficulty, (*a*) by the number of fibres involved, and (*b*) by the frequency of impulses set up by continuous or repeated stimulation. But it is well known that there is a wide range of reflex response to single stimuli as well. Sherrington and Sassa (8) have shown extensive gradation in the case of the flexion reflex in response to single shocks, all of which were of such strength that when applied to a motor nerve they provoked maximal contractions in the muscle. If one could judge from this response to motor nerve stimulation, it would be inferred that at all the strengths of stimulus under consideration every fibre in the nerve was being excited, and it would seem altogether probable that this would be the case whether the nerve was motor or sensory. If then the stimuli, which in Sherrington's experiments produced reflex responses of graded magnitudes, were all supramaximal, wherein could their differences in strength avail to produce differences in response?

The same question has been raised by Forbes and Gregg (9), and

an answer proposed which was suggested by the records of action currents set up in a nerve by powerful stimuli. Apparently a single break shock of great strength was able in many cases to set up two successive nerve impulses, appearing in the galvanometric record as two distinct action currents. Garten (10) has published similar action current records and has interpreted them in the same way. Experimenting with the mammalian sensory nerve (saphenous) we have obtained many records of double responses to strong break shocks. The most striking series is that reproduced in Fig. 12 (p. 321) from the saphenous nerve of a rabbit. Such repetitive discharge of impulses provides a possible mechanism for grading the reflex effect in response to graded single shocks whose strength is greater than is required to excite every fibre in the nerve.

Sherrington's recent work (11) has brought to light several new facts of great interest which are, at first sight, rather difficult to reconcile with this suggestion. He has measured the magnitude and duration of mechanical responses to both reflex and motor nerve stimulation, and found with the latter a well defined maximal value of the muscular twitch which, as the strength of induction shocks increased, was not exceeded except in the case of extremely powerful shocks with which a "supra-maximal" response was obtained. When the same muscle, however, was reflexly excited by single shocks the increase in height of contraction appeared to be more gradual, and continued to increase till it was greater than the maximal simple twitch of muscle, this greater value being obtained with a strength of stimulus considerably less than the powerful shock required to produce a "supra-maximal" response in the case of motor nerve stimulation. Added to the increase in the height of contraction was an increase in duration in the case of the reflex which did not appear in the case of motor nerve stimulation.

It appeared to us that an analysis of the electrical response of the muscle and of the afferent side of the arc might help to interpret these results. We have therefore measured the electrical and mechanical response of a muscle stimulated both reflexly and by way of its motor nerve by break shocks of different strength, and we have measured also the electric response of the afferent nerve to the same stimuli.

Response of different components of the reflex arc. Method. The following procedure was carried out in three experiments. A cat was anaesthetised and the spinal cord transected at about the level of the last thoracic vertebra. Decerebration was then performed at the level of the anterior colliculi by the guillotine method. The popliteal nerve

was severed in the popliteal space and stimulating electrodes were applied to the central end. The tendon of the tibialis anticus muscle on the same side was attached to a thread leading to a muscle lever which threw a shadow on the film used to record the electrical responses. A spring torsion level of high periodicity was used, but unfortunately its records showed considerable oscillations owing to the great distance between the animal and the lever and to the weight and extensibility of the thread joining them. Two windows were cut in the skin over the tibialis anticus muscle and non-polarisable electrodes connected with the galvanometer were applied at these points. Contact was made and secured by means of twine soaked in Ringer's solution, imbedded at one end in a tube of gelatine and Ringer's solution, and at the other end tied to the surface of the muscle by means of a suture passing through the fascia. A series of records was then made of the action current of the muscle together with the contraction simultaneously recorded on the film. Single break shocks were used except in a few experiments in which both make and break shocks were used in rapid sequence. A rest of several seconds was allowed before each stimulus, in order to avoid the modifying effect on the centre of a stimulus having occurred within less than a second (12). The strength of break shocks was varied from the threshold to 100 or 200 times this value. After the complete series of reflex responses had been recorded the motor nerve was cut at the hip and stimulating electrodes were applied to it. Then the responses of the muscle, both mechanical and electrical, to a similar series of stimuli thus applied to the motor nerve (peroneal) were obtained. Finally, the afferent nerve was removed to a moist chamber with the stimulating electrodes as originally applied still in place: it was connected with the galvanometer, and its action currents in response to a similar graded series of stimuli were recorded. (In one experiment the afferent nerve had become damaged and no action currents were obtainable.) In the third animal the whole procedure was repeated on the other side, except the recording of the action currents of the afferent nerve.

The strength of the break shocks was measured in the way given on p. 304, the coil distance being kept constant and the resistance of the primary circuit noted. When, however, extremely strong stimuli were desired, the coil distance was decreased from its standard value, the resulting increase of the strength of stimulus being determined afterwards by the threshold method of v. Fleischl, as described by Martin (13).

Results. The results of the four experiments (counting the left side of the third animal as a separate experiment) were all concordant in

yield when plotted, the same sort of curve as those for the muscle, and the "plateau" is reached more nearly at the strength of stimulus at

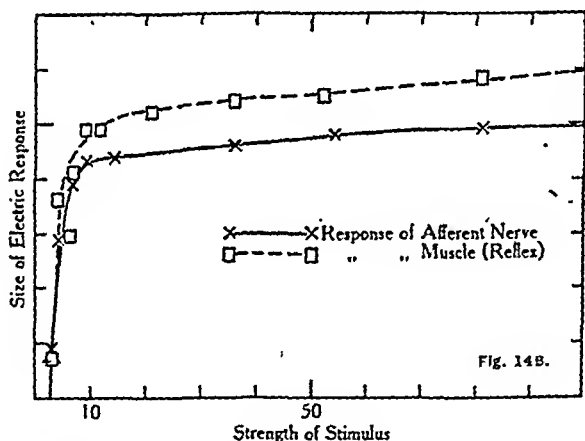
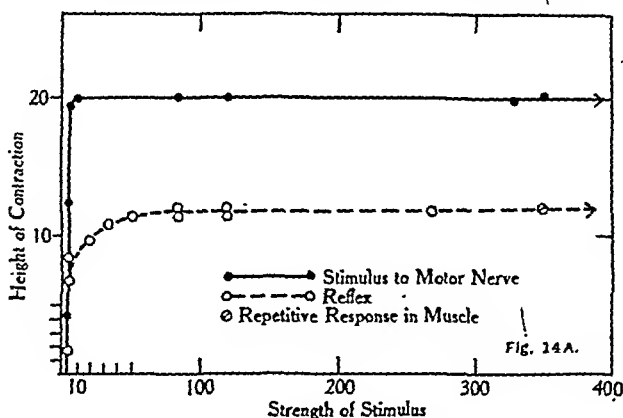


Fig. 14 A and B. Grading of response in flexion reflex (tibialis anticus).

which it is reached in the case of the reflex than in the case of motor nerve stimulation. It is impossible to trace the curve as far in the case of the afferent nerve action current as in the others, for with strong stimuli the excursion of the string is augmented by the electrical artefact, which distorts the measurements.

Clearly in the case of motor nerve stimulation, the beginning of the "plateau" signifies the attainment of the strength of stimulus required to excite all the motor fibres in the nerve. In the case of the reflex response the near approach to a plateau presumably depends similarly on excitation of nearly all the afferent fibres, and of all the afferent fibres if the curve becomes truly horizontal (as in Fig. 14 A). In the case of the action current of the afferent nerve itself a true plateau would un-

doubtedly signify excitation of all fibres, afferent and efferent alike. The much more rapid attainment of the plateau when the motor nerve is stimulated is no doubt due in part to the different sizes of the popliteal (afferent) and peroneal (motor) nerves and the consequent difference in the concentration of the stimulating current but it must also be due to a greater variability in the thresholds of sensory fibres, for the difference was still present (though reduced) when the slender internal saphenous nerve was used as afferent instead of the large popliteal.

The gradual increase in reflex responses with large increase in strength of stimulus after the plateau has been reached and presumably the stimulus has become adequate for all afferent fibres, now calls for consideration. Is this gradation such as might be explained by compound stimulation (*i.e.* repetitive response) in the afferent nerve?

Examination of the electrical records reveals evidence of repetitive response in the case of the strongest stimuli both in the afferent nerve and in the muscle reflexly excited. In the experiment shown in Fig 14, this evidence only appeared clearly with the strongest stimuli used, 350 units. Examples of it are shown in Fig 15 (C and D), in which the continuance

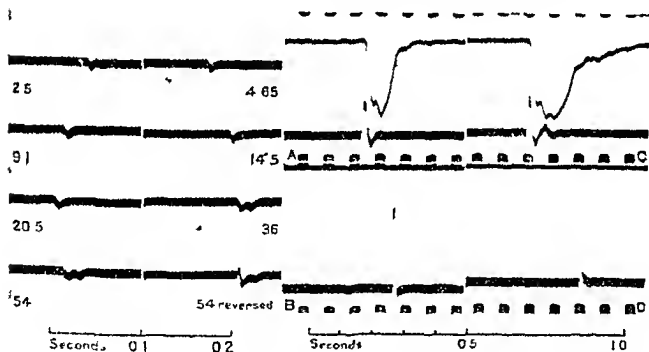


Fig 12

Fig 15

Fig 12 Action current of internal saphenous nerve of rabbit in response to stimuli of different strengths, showing repetitive response with strong stimuli.

Fig 15 A. Contraction (upper line) and action current (lower line) of tibialis anticus muscle in response to reflex stimulation of 120 units strength B Action current in afferent nerve (popliteal) 120 units C Stimulus of 320 units repetitive response in muscle D Stimulus of 320 units, repetitive action current in afferent nerve

of repeated action currents for about 0.2 second is evident in both afferent nerve and reflex muscle records. With these are contrasted in the figure corresponding records in the same experiment with a stimulus strong enough to evoke a response in each case on the plateau (*i.e.* maximal) but not strong enough to cause repetitive response. In the experiment made on the opposite limb of the same animal, repetitive discharge was found in the records of reflex muscle action current with all stimuli of over 50 units, and once with only 36 units. It is notable that the reflex responses (both mechanical and electrical) were consistently greater in this particular experiment than in any of the others. This is interesting in connection with the unusually small strength of stimulus at which repetitive reflex discharge appeared, and in connection with the comparison between our results and Sherrington's, to be discussed presently. Unfortunately, we made no record of the action currents in the afferent nerve of this limb.

In none of our records was there evidence of repetitive electric response in the muscle to motor nerve stimulation. It should be noted that none of the break shocks in our series were strong enough to evoke the well defined "supramaximal" contraction of the muscle to motor

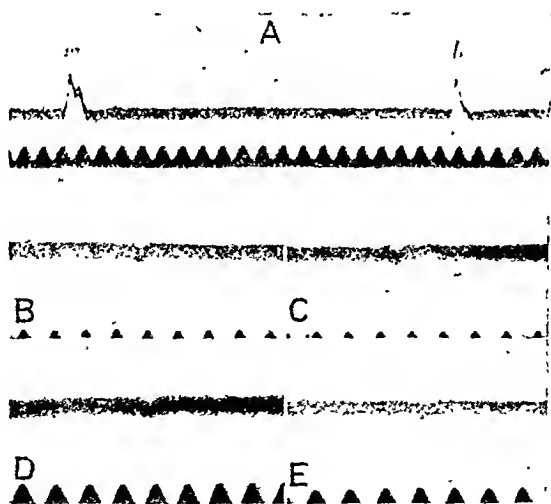


Fig. 16. A. Action current of external interosseous muscle, stimulus to motor nerve (395 Z units). Repetitive action current on make *m*, but not on break *b*. B-E. Popliteal tibial nerve muscle preparation, action current of nerve. B. Response to single stimulus. C. Double response to two stimuli at 0.0086 sec. interval. No muscular summation. D. Stimuli at 0.0095 sec. Muscular summation. E. Double response to single break shock of 630 Z units strength.

nerve stimulation, which Sherrington illustrates in Fig. 6 of his recent paper (14). Make shocks of great strength from a Berne coil with an iron core, have been found by one of us to cause when applied to the popliteal nerve a repetitive electric response in the extensor interosseus muscle of the foot (Fig. 16 A), although in the same experiment break shocks failed to do so. This is doubtless to be explained by the fact that make shocks last much longer than break shocks.

We may conclude from a survey of our experiments that the first and obvious source of gradation in reflex response to single shocks depends on the number of afferent fibres excited, and this stage of gradation corresponds with the initial rise in the curve before the "plateau" is reached. Any further increase in the size of response appears to consist in the appearance of repetitive discharge in the motor neurones, as Sherrington has inferred. In all but the last of our experiments the strength of stimulus required to evoke this repetitive reflex response seems to have been such as was also shown to set up repetitive discharge in the afferent nerve fibres.

Repetitive discharge of central origin. It will be interesting now to make a further comparison of our results with Sherrington's. In absence of a calibration which would tell us precisely the relative strengths of Sherrington's stimuli, we may yet arrive at a rough estimate of their probable values by assuming what is true for some coils at least, viz. that the stimulus decreases in strength roughly in proportion to the square of the increased coil distance beyond a point (say 10 cm.) near where they cease to overlap. Plotting a curve of the maximum heights attained in the myograms in Fig. 1 of Sherrington's paper against provisional units so obtained, gives a curve which rises rapidly at first and then much more slowly and does not differ from our curves as much as might be expected. But there is still a most important difference. Whereas we found reflex responses rarely exceeding in magnitude the maximal "neuro-myal" twitch of the muscle, and never exceeding it by a large amount, Sherrington found even at the beginning of this "plateau" a reflex response more than twice as large as the maximal twitch. This effect he ascribes to repetitive discharge in the motor neurones, and indeed there appears to be no other possible cause for it.

The most interesting feature of this result is that the repetitive motor discharge from the centre occurs in response to a single shock which is presumably too weak to produce repetitive impulses in the afferent nerve. The presumption that these stimuli of Sherrington's were too weak

to cause compound response in the stimulated nerve, is strengthened by our last experiment in which, as already noted, we regularly obtained repetitive electric responses in the muscle when reflexly excited by stimuli much weaker than we ordinarily found necessary to cause repetitive response in the afferent nerve. It is most unlikely that with 36 units we set up a double response in the afferent fibres, yet this stimulus sufficed to evoke a repetitive discharge from the centre. It is a striking fact that this type of discharge should result from a single simultaneous volley of afferent impulses. Our experiments indicate that in some preparations this does not occur: and the spinal centre evidently varies a good deal in its ability to respond with repetitive discharge to simple afferent volleys. In our last experiment, as already intimated, turning the animal over and repeating the procedures in the opposite limb was accompanied by a marked change in the size of response, and—what is more interesting—a change from a centre which could not respond by tetanus to a single afferent volley to a centre which could. Variability in the intensity and character of response to afferent impulses is a pronounced characteristic of reflex centres, even in the case of so simple and regular a response as the flexion reflex.

To account for this repetitive discharge from a centre, two different explanations have been put forward. Sherrington (*loc. cit.* p. 256) has suggested that there may be set up in the centre a sustained disturbance which might be different in kind from the transient disturbance which constitutes the impulse in a peripheral nerve fibre. We do not know enough of the nature of central activity to assert or deny the existence of such a disturbance, but there is also the possibility that the single impulse becomes multiplied because it passes into a number of dendrites or collaterals, all of which ultimately converge on the motor neurone. Unless the time spent in conduction is the same in every path a sequence of impulses will result (15).

Different effects of strong stimuli applied to motor and sensory nerves. We have already mentioned that strong single break shocks applied to the motor nerve failed to set up in the muscle more than a single response, although they frequently set up repetitive responses in the afferent nerve. At first sight this would seem to imply a great difference in the effect of a strong shock on motor and sensory fibres. This, however, is not an inevitable inference, for the strong stimulus might set up two responses in the motor nerve, although only one appeared in the muscle. Keith Lucas (16) has shown that in a frog's muscle nerve preparation it is usually possible to set up two impulses in the nerve so close together

that the second reaches the muscle at a time when it is unable to give a second response. The interval between the two stimuli can only vary within narrow limits if the effect is to be obtained, and it is sometimes absent altogether (17). But if two impulses are set up by a strong single stimulus we should expect them to be separated by the shortest possible interval, *i.e.* an interval only just longer than the absolute refractory period of the nerve, and with this interval the muscle might well be unable to respond to the second impulse.

To determine whether a similar effect occurred in a mammalian muscle nerve preparation proved a difficult matter because of the necessity of working at body temperature and of avoiding electrical artefacts. A method which has finally proved successful is as follows.

In a decerebrate cat the popliteal branch of the sciatic nerve was severed at the hip and dissected from the rest of the sciatic trunk for about 3 cm. in the thigh. Through a second incision in the popliteal space the nerve was again exposed and all muscular branches given off in this region were severed. The continuation of the nerve (tibial) was then exposed in the lower leg and dissected from the surrounding tissues for about 6 cm. Stimulating electrodes of the Sherrington shielded type in a glass tube were applied to the cut end at the hip. The proximal end of this tube was sealed with wax to prevent escape of current at this end. The thigh incision was then sewed around the neck of the tube in order to keep this portion of the nerve at body temperature. A second glass tube with a slot in the side was placed in the lower limb in such a way that two non polarisable electrodes of the silver chloride type could be applied to it 16 mm. apart inside the glass tube and the nerve could be insulated from the surrounding tissues for the distance of 5.5 cm. This proved necessary to prevent the surrounding tissues from short circuiting the action current. The skin was then sewed around the opening of the glass tube. In this way diphasic action currents could be recorded with the second phase following so close upon the first that the first phase appears as a sharp spike in the galvanometric record. The external interosseous muscle in the foot (innervated by this nerve) was exposed on its ventral aspect and leads were applied: the "proximal" to the middle of the muscle, the "distal" to its proximal end at the heel, for the reason that the propagated disturbance in this muscle was found to travel toward the heel. By means of a double pole double throw switch the galvanometer could be connected with either the nerve or the muscle. A thread tied around the toes was connected with a light bell crank lever in such a way that the contraction of this muscle could be recorded on a smoked drum. Two stimulating coils were connected with a Lucas pendulum capable of delivering two break shocks at intervals which may be determined accurately to within ± 0.00003 sec. The secondary coils were connected in parallel, and both connected with the stimulating electrodes so that the cathode on the break shock should in each case be distal.

It proved easy to tell with this arrangement whether a second response occurred in the muscle, by Boycott's method of noting whether the record on the smoked drum showed summated contraction or not. The electrical response of the muscle provided an additional check on this method. In two separate experiments with the external interosseous muscle, and in one similar experiment with the peroneal nerve and tibialis

anticus muscle, it has been found possible by proper timing of the second stimulus to obtain electrical records showing two responses in the nerve when the absence of summated contraction showed that there was only one response in the muscle. In each experiment this observation was made repeatedly. Freedom from confusion due to electrical artefact in the case of the popliteal nerve was shown in the following way: When the experiment had been completed the nerve was crushed a few millimetres distal to the point of stimulation to prevent impulses reaching the leads. Galvanometric records were then made while the strongest stimulus employed was applied to the nerve. The absence of any discernible excursions of the string sufficed to show that the well-defined excursions in the previous records had been due to action currents, not artefacts. Fig. 16 B-D, shows an example of double action currents in the nerve when only a single response occurred in the muscle. For comparison are shown also the response of the nerve to the first stimulus by itself and the double response when the second stimulus was so timed as to evoke a second response in the muscle.

In one of these experiments we found evidence bearing more directly on our question, viz. as to the possibility of repetitive impulses in the motor nerve in response to a single shock applied directly to it, failing to set up more than one response in the muscle. Fig. 16 E shows the record of the electrical response of the nerve to a single break shock of great strength. It is from the same experiment as the records in Fig. 16 B, C and D, and was taken immediately after them without in any way disturbing the apparatus or connections. It appears to reveal quite clearly a second nerve impulse. The contraction of the muscle was no more than a simple maximal twitch.

We may therefore conclude that the failure of a muscle to show repetitive response to single powerful shocks applied to its motor nerve, does not necessarily mean failure of the motor nerve itself so to respond. This conclusion tends to eliminate an apparent difference between sensory and motor nerve fibres. There is, however, a possibility which must not be overlooked. The peroneal and popliteal nerves contain both sensory and motor fibres: and it is conceivable that in the above experiments the double response only occurred in the afferent fibres, and that the motor fibres never responded twice without causing the muscle to respond. We see no reason to suppose this is so, but further evidence is needed to exclude the possibility completely.

is us:

III. REMARKS.

The preceding experiments have shown that the sensory nerve fibre reacts on the all-or-nothing principle and they have confirmed the suggestion of Forbes and Gregg that the wide variation in reflex response to single stimuli is due to the setting up of more than one impulse when the stimulus is strong. This conclusion may not be true of all the fibres of a sensory nerve trunk, but it is at any rate true of the majority. That the same result is given by mammalian sensory nerves and by the motor nerves of the frog suggests that all medullated nerve fibre may be expected to react in the same way. The importance of this result for theories of hearing and vision is self-evident, though its extension to the optic and auditory nerve fibres cannot be regarded as absolutely certain. In the case of the optic nerve, however, Troland (18) has already brought forward some evidence for an all-or-nothing reaction, and Jolly (19) has shown that it is not incompatible with a photo-electric theory of vision.

It is possible that a sensory nerve may contain a few fibres which do give a graded reaction by conducting with a decrement. Non-medullated sensory fibres, if such exist, would probably conduct in this way, for Garten's work suggests that this is the normal type of conduction in non-medullated nerves. The presence of a few such fibres might account for the failure to reach a steady plateau in the action current of the afferent nerve or in the reflex response in some of our experiments. On the other hand we do not think that more than a few fibres of the internal saphenous can conduct in this way, since we found no signs of decremental conduction in the isolated nerve trunk. On general grounds we might expect that decremental conduction would not occur except to a trifling extent in the nerve fibres of vertebrates, since it would mean that reflex arcs with a short conducting path would be more effective than arcs with a longer path. In primitive animals with a diffuse nerve-network it may be an advantage for the reflex response to be most intense in the neighbourhood of the point stimulated, but such an arrangement would obviously not be adapted to the needs of the more complex organisms in which there is a central nervous system connected with the periphery by fibres of variable length. With such an arrangement it would seem almost essential that the impulse should be conducted without change of intensity in the peripheral nerve fibres.

Two other points deserve mention. The first relates to the identification of the nerve fibre as the ultimate conducting unit. We have assumed

throughout that the nerve fibre is such a unit and that it is not a bundle of neuro-fibrils each capable of independent activity. This assumption has been discussed elsewhere⁽²⁰⁾: it is based on the observation of Keith Lucas⁽²¹⁾, that a muscle supplied by 10 nerve fibres but containing 150-200 muscle fibres shows not more than 10 different strengths of contraction in response to a continually increasing stimulus. We would point out, however, that the conclusions arrived at would not lose their force even though the ultimate unit were the neuro-fibril and not the nerve fibre. There would be a greater opportunity for the gradation of reflex effects by variation in the number of units in action, but it would still be impossible to set up impulses of different intensity under similar conditions and this would involve much the same consequences in regard to the central mechanism and to the nature of the nervous impulse.

The other point concerns the meaning to be attached to the statement that the nerve fibre reacts on the all-or-nothing principle. This phrase is sometimes used as though it meant that every impulse passing down the fibre must have the same intensity whatever the conditions of its propagation. There is abundant evidence that the nerve fibre does not react in this way and we have used the phrase to mean, not that the intensity is invariable, but that it depends only on the local conditions at the point at which it is measured. The intensity of each impulse will vary with the state of recovery of the fibre and therefore with the frequency of stimulation (within limits), but if the nerve gives an all-or-nothing reaction the intensity of the impulses cannot be altered independently of the frequency.

CONCLUSIONS.

1. In a motor nerve fibre under normal conditions the impulse follows the all-or-nothing principle. This does not hold in a region of decrement where the size depends on the distance the impulse has travelled, and if an impulse is set up in such a region its size will vary within limits with the strength of the stimulus. In this our experiments confirm those of Lodholtz and Rehorn.

2. If the decrement in conduction in unit length is small, there is little variation in the initial size of the impulse with stimuli of different strength; a wider variation is possible if the decrement in unit length is large.

3. A mammalian sensory nerve (the internal saphenous of the cat)

shows no clear evidence of a decrement in conduction when tested by two different methods. Thus very little gradation in the initial size of the impulse will be possible in sensory fibres unless there is some fundamental difference between motor and sensory fibres.

4. Impulses set up in the internal saphenous by stimuli of different strength are all equally capable of passing through a narcotised region, and when conduction fails for an impulse set up by a weak stimulus it fails also for a strong stimulus. The size of the impulse is therefore independent of the strength of the stimulus in the sensory as in the motor fibre.

5. The response of a sensory nerve trunk to a single, momentary stimulus may vary in two ways, (a) a strong stimulus will excite more fibres than a weak, and (b) a stimulus which is more than strong enough to excite all the fibres may set up two or more impulses in each fibre.

6. The response to stimuli of different strengths has been measured in different parts of the arc which is concerned in the flexion reflex in the spinal cat. With reflex stimulation the response of the muscle agrees very closely with that of the afferent nerve, and the gradation seems to depend on (a) the number of nerve fibres stimulated, and (b) the repeated excitation by strong stimuli.

7. When the motor nerve is stimulated the muscle does not give more than a single maximal twitch although the stimulus may be strong enough to give a double response in the nerve. Probably the second impulse has no effect because it reaches the muscle at a time when the latter is still in the absolute refractory state. In the reflex arc a second impulse due to strong stimulation of the afferent nerve has more chance of affecting the muscle owing to delay at various synapses, etc.

8. A single impulse in the afferent nerve may sometimes evoke two or more impulses in the efferent side of the arc. Whether it does so or not depends on the condition of the spinal centres.

9. In general the reactions of the reflex arc support the view that the large majority of sensory fibres react according to the all-or-nothing principle.

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THE EFFECT OF SECTION OF THE VAGI ON THE RESPIRATION OF THE CAT.

BY J. TREVAN AND E. BOOCK.

(From the Wellcome Physiological Research Laboratories.)

ROSENTHAL(1) was the first to point out that the effect of section of the vagi was modified by the transection of the brain at different levels. He showed that a section through the brain stem at the level of the posterior border of the posterior colliculi, followed by division of the vagi, resulted in such profound modifications of the respiratory response, that the animal dies of asphyxia in a short while, although before cutting the nerves the respiration is not very conspicuously different from the normal. These experiments were repeated and extended in 1916 (Trevan(2)). The respiratory centre is considerably modified in its response to other changes when the posterior colliculi are removed. It is, for example, less sensitive to the effect of change in the reaction of the plasma (Trevan(2)), and it is no longer stimulated by the injection of acetyl acetone and similar substances (Hurtley and Trevan(3)). The usual effect of section of the vagi in an anæsthetised animal is a much less profound change in the respiratory movements, whilst Schafer(4), on cats and rabbits, has shown that if an animal is allowed to recover from the anæsthetic, the effect on the normal rate and depth of the respiration on section of the vagi is very slight, provided laryngeal palsy is prevented. Schafer gives also a large number of experiments to show that anæsthetised animals do not constantly or often give the classical results.

The following experiments were made in an attempt to correlate in some degree these different actions.

Decerebrate cats. Cats, anæsthetised with ether and tracheotomised, were decerebrated with Sherrington's guillotine and the remainder of the cerebrum was removed with a scalpel. The carcase was then left

for periods of three to six hours, in order to recover from the shock. In our experience, the respiratory centre is working at its best between the sixth and the tenth hour after the operation; the carcase should be untouched for at least three hours for even gentle handling before this time may considerably modify the response of the centre. The respiration was recorded by a string stitched to the abdominal wall and run over a pulley to a lever; in the tracings down stroke is inspiration.

If the section of the brain stem is made at level I in Fig. 1, respiration

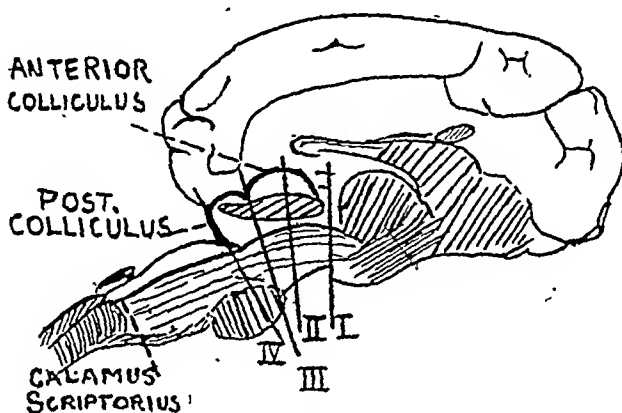


Fig. 1.

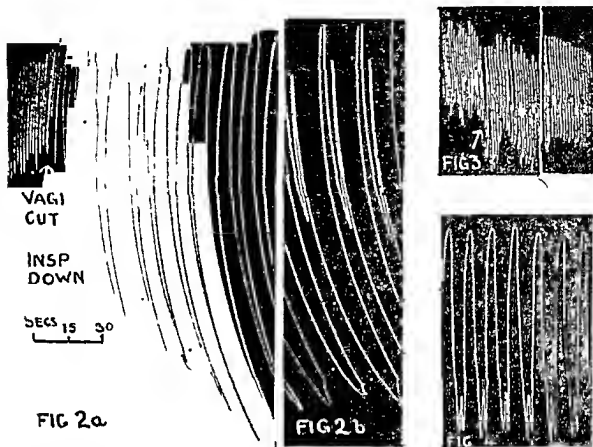
may be slightly deeper and slower than normal, but it is not seriously altered. If the section is behind this point, deepening becomes more marked and corresponding slowing appears, the depth usually continually increasing, the further the medulla is encroached upon.

The response of the centre to section of the vagi depends on the level of the section through the brain stem.

Section of the vagi in a cat decerebrated at level IV causes profound alteration, similar to that recorded by Rosenthal. The respiratory movements consist of a series of inspiratory gasps with pauses in the inspiratory position—a sort of vagal ataxia. A record is shown in Fig. 2. About 20 minutes after the termination of the tracing, the animal died of asphyxia, the rate having become much slower, the inspiratory standstill much longer. Death may be delayed for half an hour, but rarely longer. The differences are further illustrated by the following measurements:

	Depth	Rate	Minute volume
Before division of vagi	17	27	459
After " "	48	6	288
After (maximum) " "	70	6	420

If the section passes through the anterior colliculi (level II) the effect of the division of the vagi is much less marked. There is slight slowing



Figs. 2, 3, 4. Effect on respiration of section of the vagi after decerebration. Fig. 2. Decerebration at level IV of Fig. 1. The interval between a and b was 20 mins. Fig. 3. Decerebration at level II. Both vagi divided at the arrow. Gap in the tracing about 3 mins. Fig. 4. Decerebration at level III. The abdominal movement precedes the thoracic.

and compensatory deepening, shown in the tracing given in Fig. 3. Measurements of the tracing give the following results:

		Depth	Rate	Minute volume
Before section	...	21	28	588
After	„ ...	24	23	552

If the section passes between the colliculi (level III), division of the vagi gives a greater deepening of the respiration, and a curious in-coordination of the respiratory movements usually shows itself at this stage, especially, and sometimes only, on increasing the dead space of the tracheal cannula. It consists of a time dissociation of the abdominal and thoracic respiratory movements. It is illustrated in Fig. 4. The inspiratory movement is downwards, and the first wave on the curve at A was found by inspection at the time of the experiment to be

Fig. 7 shows the effect of ether on the respiratory rate of the cat. The depth of anæsthesia was varied by increasing the amount of ether inhaled. As the corneal reflex disappeared it will be seen that the respiration became shallower and quicker. If the ether administration is continued, the shallowing becomes very marked, and the respiratory movements stop. If the anæsthesia is not allowed to go so far as to stop the respiratory movements, the respiration deepens with diminution in the anæsthesia, and the original depth and rhythm were restored. If the very deep anæsthesia is maintained for any length of time, the lightening of the anæsthesia sometimes results in the establishment of a vagal type of respiration, although the vagi are intact.

Fig. 8, top tracing, shows the effect of section of the vagi in a cat



Fig. 8

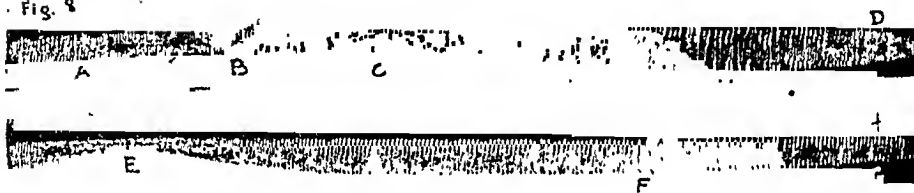


Fig. 7. Increase of ether with intact vagi. Corneal reflex present, $CR+$; absent, $CR-$. Ether increased, $E+$.

Fig. 8. Effect of ether with vagi cut. See text. D , corneal reflex returned. Lower tracing, 45 mins. later. Deeply etherised at left end of tracing, lightly at right end.

anæsthetised with ether. The animal with one vagus cut already being deeply anæsthetised, the ether was increased at A . Then the second vagus was cut at B , and there was a slight deepening of the respiratory movements. Almost immediately afterwards the rapid shallow breathing foreshadowing respiratory failure from the anæsthetic set in, and the ether was taken off at C . This was followed quickly by deepening and slowing of the respiration till the rate went down to half what it was before the vagi were cut; there was a marked pause in inspiration at this stage. Then, as the anæsthesia became still lighter, the original rhythm returned. The whole cycle was repeated five times, and Fig. 8, bottom tracing, gives such a cycle obtained 45 minutes after the first. In each cycle vagal breathing appeared just about the time that the corneal reflex

disappeared, and vanished when the anæsthesia became very deep. The variation in rate is plotted in Fig. 9, with an arbitrary representation

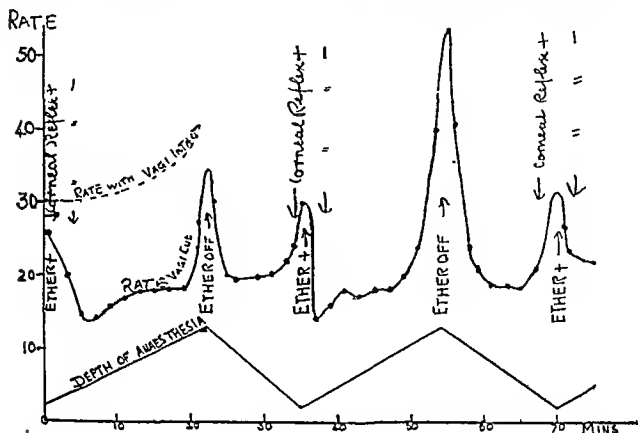


Fig. 9. Effect of varying depth of anæsthesia on rate of respiration after section of vagi. The top curve represents alteration of rate. The bottom one is an arbitrary representation of "depth of anæsthesia."

of the depth of anæsthesia. These results have been obtained in other cats, and, provided the animal is not kept very deeply anæsthetised too long, vary only in degree. The corneal reflex is not always a good index of the depth of anæsthesia, and the diminution in depth produced by the anæsthetic comes on more easily in some animals, and tends to mask the effect of vagotomy. This is practically always deepening of the respiration, although the inspiratory pause may not occur.

If, however, the animal is kept for any length of time in the deeply anæsthetised state with the vagi cut, the return of the respiration to normal does not take place for a considerable time after the corneal reflex has reappeared.

Remarks. The importance of the part of the respiratory centre above the medulla pointed out by Rosenthal is emphasised by these experiments. We have not completely worked out the position of the nervous arcs involved. They are not actually in the colliculi, for in removing the anterior part of the brain stem, after the first stage of decerebration, interference with respiration only takes place when the ventral part of

ON THE CORONARY CIRCULATION IN THE HEART-
LUNG PREPARATION. BY TOMOICHI NAKAGAWA,
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In an investigation by Markwalder and Starling⁽¹⁾ on the factors which influence the blood flow through the coronary arteries, it was shown that in the increase of blood flow through the heart muscle which accompanies increased activity of the heart, three factors were involved. These were (1) rise of the arterial blood-pressure, (2) stimulation of the sympathetic nerve, or the presence of adrenalin in the blood, (3) the production of metabolites by the heart muscle, when for any reason its supply of oxygen falls short of its requirements. Carbonic acid and lactic acid had an influence in the same direction but were not capable of producing anything like the dilatation of the coronary vessels which could be brought about by these unknown metabolites. These facts were also observed by Morawitz and Zahn⁽²⁾, working on the heart *in situ*.

A rise of arterial pressure is not, however, the only factor which may necessitate increased effort on the part of the heart muscle. The consumption of oxygen by the heart is increased under all conditions which evoke increased mechanical work. Under the conditions of the heart-lung preparation it is possible to increase the inflow and the output of the heart without altering in any way the arterial blood-pressure, although the work of the heart muscle and the dilatation of the heart, and therewith the chemical changes, are largely increased. It was shown, moreover, by Evans⁽³⁾ that the oxygen usage of the heart—the resistance being kept constant—is nearly constant per beat, so that the consumption of oxygen per minute is almost proportional to the number of beats. The heart requires more oxygen therefore when its rhythm is increased by artificial stimulation, by rise of temperature, or by removing any tonic action of the vagus. It seemed interesting to enquire how far these conditions influenced the flow through the coronary arteries, and whether, in fact, the automatic regulation of the coronary circulation is so perfect that it reacts by an increase to any factor which raises the oxygen consumption of the heart muscle. At Prof. Starling's suggestion I have

therefore undertaken the investigation of these questions. I have also made some experiments on the influence of pericardial effusion, or rather of the presence of fluid in the pericardial sac, on the flow through the coronary circulation.

Methods. Most of the experiments were carried out on medium sized dogs. These were anæsthetised with morphia and C.E. mixture and were then given an intravenous dose of chloralose, .1 gm. per kilo. A heart-lung preparation was made as described in Knowlton and Starling's paper(4) and in subsequent papers from this Institute. The arterial pressure was measured by a mercurial manometer which was connected with the side branch from the arterial cannula. From the venous end of the apparatus the blood was allowed to flow into a graduated cylinder, and the time taken to collect 50 c.c. determined by means of a stop-watch. This amount was the arterial output, *i.e.* the output of the heart minus the blood flowing through the coronary arteries. The coronary outflow was measured by collecting the blood through a Morawitz cannula(5) introduced into the coronary sinus, and the time taken to collect 10 c.c. was noted. The blood flowing through the cannula was returned to the venous reservoir every few minutes, but it was necessary to circulate fresh amounts of blood to remove the effect of vaso-dilator metabolites. In each experiment it was found necessary to use blood from another dog, or in some cases from two dogs. The blood was defibrinated.

In order to investigate the influence of the pericardial fluid on the coronary circulation, de Baronne's(6) heart-lung preparation with empty beating right heart was used. After Starling's heart-lung preparation was made, a small incision was made in the pericardium just above the pulmonary artery, into which a T-shaped cannula was inserted. One branch of the T cannula was connected with the rubber tube to the venous reservoir, which was raised to the height of a column of blood of 50 cm. The third branch of the cannula was stopped by a rubber cork with a thermometer. The blood flowing into the pulmonary artery reaches the left side of the heart through the lung, so that the right heart receives only the whole blood through the coronary vessels. This blood was collected through a cannula inserted in the superior vena cava, and the time required to collect 25 c.c. was taken. The scheme of this circulation will be seen in de Barenne's paper(7). The opening made in the pericardium was then sewn up to prevent leakage of the fluid injected into the pericardial cavity.

In the tables the c.c. of blood are given to the nearest whole number.

It is stated by Starling and his co-workers that the output of the heart is practically independent of the temperature within wide limits, and in the above experiments, therefore, the systemic output was sometimes measured. The venous inflow given in Table II was calculated by adding to the systemic output the mean value of the calculated coronary outflow.

The influence of the rate of heart beat on the coronary circulation. In endeavouring to interpret the above experiments it was necessary to eliminate the direct action of temperature on the coronary circulation from the indirect one which might be brought about mechanically by the alteration in the rate and amplitude of heart beat also induced by temperature alterations. Accordingly an endeavour was made to alter the rate of heart beat while maintaining the temperature constant.

In order to get tachycardia the sino-auricular node was stimulated by means of single induction shocks. Any fall of the arterial pressure caused by the stimulation was prevented by adjustment of the arterial resistance, the mean arterial pressure being maintained constant. Before and during the stimulation, the temperature, the venous inflow and the pressure were the same. The following table shows the effect of tachycardia on the coronary circulation.

TABLE III. Effect of acceleration of heart beat.

Weight of dog. Kilos.	Systemic output c.c. per min.	Arterial pressure mm. Hg.	Temp.	Heart beats		Coronary output c.c. per min.		Secs. of stim.
				Before	During	Before	During	
11.0	306	90	30	102	132	50	50	24
"	"	"	29	84	132	51	51	23
"	"	"	28	84	132	47	48	21
"	"	95	35	126	132	54	54	16
10.0	312	90	28	84	120	45	45	13
11.0	375	"	33	120	168	34	34	17
"	"	"	31	104	168	36	36	—
9.0	480	90	37	156	186	33	33	8
10.5	320	"	31	84	138	64	63	9
9.5	282	95	31	96	162	69	67	9
"	"	"	"	"	"	69	68	—

Thus acceleration of the heart beat has little, if any, influence on the coronary output. Morawitz and Zahn(2) found that the tachycardia caused by warming the sino-auricular node diminishes the coronary outflow, but in their experiments the diminution of the coronary output was accompanied and was probably caused by a fall of arterial pressure. In Table III it will be seen that when the node was stimulated for a short period, the coronary outflow was slightly decreased, but when the time

of stimulation was longer, the outflow was the same before and during the stimulation. In conclusion it may be said that tachycardia itself has no influence on the coronary circulation, which is thus apparently independent of the rate of the heart beat.

In order to obtain slowing of the heart beat the peripheral end of the cut vagus nerve was stimulated with an alternating current. The consequent fall of arterial pressure was adjusted by raising the arterial resistance so that the pressure was maintained constant. The venous inflow and the temperature of the blood flowing to the heart were also kept constant before and during stimulation. The results are given in Table IV.

TABLE IV. Effect of slowing of heart beat.

The arterial pressure in each experiment was kept at 90 mm. Hg.

Weight of dog. Kilos.	Temp.	Systemic output c.c. per min.	Heart beats		Coronary sinus		Secs. of stim.
			Before	During	Before	During	
10.8	33	270	126	30	21	21	27
8.7	34	204	120	40	20	20	17
				31	20	10	20
9.0	37	500	156	23	28	29	12
	38	481	162	55	29	33	5
7.4	36	178	163	51	23	22	11
10.0	35	125	126	60	30	30	18
					30	32	17
8.0	30	190	108	50	23	24	12
"	"	"	108	65	24	25	9

It will be seen that the coronary outflow in most experiments was unaffected by the slowing of the heart produced by vagus stimulation, but occasionally there was a slight increase or decrease. At the beginning of stimulation the heart acts forcibly, takes up and drives on more blood, and if the outflow is calculated as that occurring during the first few beats it is sometimes found to be considerably increased. Notwithstanding that during the period of slowing as a whole, the circulation through the heart is retarded, there does not appear to be sufficient accumulation of metabolites to cause vaso-dilatation.

The influence of pericardial effusion. In investigating this point, de Barenne's method, namely, leading the blood from the venous reservoir directly into the pulmonary artery, was employed. The effects of pericardial effusion were imitated by allowing liquid paraffin at about 30° C. to flow into the pericardial sac. Considerable difficulty was met with in preventing leakage from the opening which had been previously made and sewn up for the purpose of inserting the cannula into the pulmonary artery. In the following experiment the leakage was, however, very slight.

OBSERVATIONS ON THE MELANOPHORES OF THE FROG. BY K. UYENO, M.D., *Tokio.*

(*From the Physiological Laboratory, Cambridge.*)

Effect of CO₂ and O₂ on the cutaneous melanophores.

THE method I have followed in investigating the effect of CO₂ and of O₂ on the cutaneous melanophores is to cut off the legs of a frog immediately after death, to place one in 0.6 p.c. NaCl solution in an atmosphere of O₂, the other in the same solution in an atmosphere of CO₂, and to observe from time to time the state of the melanophores in the webs. The following experiment will serve to illustrate the effect when the pigment cells at the time the frog is killed are about midway between complete dispersion and complete concentration of the granules.

Exp. 1. March 27. Frog killed at 3 p.m. Medium concentration of pigment in each foot.

March	Right foot	Left foot
27 3 p.m.	Put in O ₂	Put in air
3.55	Complete concentr.; put in air	Slight concentr.
5	Put in CO ₂	Complete concentr.; put in CO ₂
28 11.10 a.m.	Moderate dispersion; put in air	Moderate dispersion; put in O ₂
11.55	Slight concentr.	Half of cells complete concentr.
2.20 p.m.	Majority moderate dispersion, few strong concentr.	Majority complete concentr., some moderate dispersion; put in CO ₂
5.10	No further change; put in O ₂	Moderate dispersion, less than before
29 10.30 a.m.	Complete concentr.; put in CO ₂	Complete concentr.; put in CO ₂
3 p.m.	No change	No change

It will be seen that in the foot placed in O₂ there was complete concentration of pigment in less than an hour, and that transference to CO₂ caused moderate dispersion in most of the cells lasting many hours. In the foot placed in air complete concentration took two hours, CO₂ then caused moderate dispersion, O₂ again complete concentration, and CO₂ once more some dispersion but less than before. The final concentration is no doubt an accompaniment of the death of the cells. The time relations of the changes vary in different experiments. Exp. 2 shows some of these and also the effect when the granules were completely dispersed at the time the frog was killed.

Exp. 2. March 29. Frog was killed at 3.30 p.m. Complete dispersion of pigment in each foot.

March		Right foot	Left foot
29	3.40 p.m.	Put in O ₂	Put in air
	4.20	Complete concentr. in most areas	Trifling concentr. in whole area
	5	Concentr. more extensive	Moderate concentr. in whole area
30	11.20 a.m.	Complete concentr. of all cells; put in CO ₂	Nearly complete concentr., few cells near the margin remain in moderate dispersion; put in CO ₂
	12.40 p.m.	Trifling dispersion, less than the left	Slight dispersion
	2.45	Moderate dispersion, slight, less than the left	Moderate dispersion
	5.15	Dispersion continued	Dispersion continued
31	11.25 a.m.	Fairly strong dispersion, slight, stronger than the left; put in O ₂	Fairly strong dispersion; put in O ₂
	1 p.m.	Moderate concentr.	Strong concentr.
	3.20	Complete concentr.; put in CO ₂	Nearly complete concentr.; put in CO ₂
	5.20	Slight dispersion	Distinct dispersion

Changes in deep-lying melanophores.

It is known that the deep-lying melanophores differ in their reactions from the cutaneous melanophores. So far as I know one change only has been described. Lieben¹ has stated that a slight lessening in tint of the processes is caused by adrenaline, and this he takes to show a trifling movement of the granules.

In the mass of fat, which lies just above the larynx of the frog, the pigment cells can easily be observed under the microscope with reflected light, by simply cutting the sternum in the mid-line and thrusting the cut ends sideways. These pigment cells are variable both in number and appearance in different frogs. In most cases they were found to be like the expanded² pigment cells of the web, *i.e.* they were of irregular shape and showed many processes of varying size and length; only in a few cases they showed the simpler form of the contracted² pigment cells of the web.

The marked difference between the visceral pigment cells and those of the web is that in the former the melanin granules are distributed uniformly throughout the cell-bodies and the greater part of the processes, there being no difference in the tone of colour, though marginally in the ends of the processes or in the small branches of the processes the pigment appears in particles of different sizes arranged in rows; while in the latter the pigment is not to be seen as small particles in any part

¹ Lieben. *Zntrlb. f. Physiol.* 20. p. 108. 1906.

² I use the terms, expansion and contraction of pigment cells, for convenience without prejudice to the question whether the visible change is due to the putting out and drawing in of the cell processes or to change in the position of the granules without change of form of the processes.

2. Oxygen and carbonic acid appeared to have a similar action on the deep-lying melanophores, but it was very slight.

3. The deep-lying melanophores show to a varying degree post-mortem changes. In most cases no change was observed in over 24 hours, in some distinct dispersion of the pigment and in few concentration. Even in the same frog the different cells showed different kinds of change. The post-mortem change is not obviously modified by adrenaline, and it certainly does not prevent dispersion of the pigment. Pituitrin hastens dispersion and prevents concentration. Slight inflammation has a similar effect to that of pituitrin, but to a much less degree.

Thus the deep-lying melanophores react to O_2 , CO_2 , pituitrin and mustard in the same manner as the cutaneous melanophores, but differ in their reaction to adrenaline. This difference may be explained by a lack of innervation of the deep-lying melanophores, and by the action of O_2 , CO_2 , pituitrin and mustard being direct on the cells.

I am greatly indebted to Prof. Langley for suggesting this work, and for his kind advice throughout.

ON SECRETION BY THE ISOLATED KIDNEY.

By E. B. VERNEY AND E. H. STARLING.

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THE heart-lung preparation affords a means by which any isolated organ may be fed with arterial blood of known composition at any desired pressure, rate of flow, and temperature. It is thus possible to study the functions of an organ apart from nervous influences and from the chemical influences which may arise in consequence of modifications in the blood caused by other organs of the body. The method was used by Bainbridge and Evans⁽¹⁾ to investigate the gaseous exchanges of the isolated kidney. These observers, however, did not succeed in obtaining more than a minimal flow of urine. It seemed to us worth while to take up their experiments again and to see whether it were not possible to maintain the kidney in such a normal condition that it would secrete urine in quantities permitting of an accurate chemical examination of this fluid.

Method. Two dogs were used in each experiment, one supplying the kidney, and the other being used for the preparation of the isolated heart-lung. The first dog was given morphia, anaesthetised with chloroform and ether, bled to the extent of approximately 350 c.c. and an intravenous injection of about 500 c.c. 0.9 % NaCl in tap water given immediately afterwards. A wet blanket was then placed over the animal to reduce its temperature and so reduce the oxygen consumption of the kidney.

A heart-lung preparation was then made on the second dog according to the method described elsewhere⁽²⁾ under morphia and chloralose anaesthesia. The blood obtained from the first dog was defibrinated and used for this preparation. Artificial respiration was maintained by means of a Meyer pump, the inspired air being saturated with moisture by passing it through a jar of warm water. A \perp -piece in the arterial tube of the heart-lung preparation side-tracked part of the blood to a cannula suitably chosen to fit the renal artery. Immediately proximal to the cannula was a screw clip so that a slow stream of blood could be allowed to escape during the insertion of the cannula. The abdomen of the first dog was then opened. A small glass cannula was placed in the left ureter and this divided below. The kidney was freed from its bed and a ligature

passed round the renal artery and loosely tied. The aorta was clamped immediately distal to the artery and divided below the clamp. A small incision was made into it immediately opposite the origin of the renal artery, a clamp applied to the aorta just above this, and the aorta, renal vein and any remaining structure quickly divided. The kidney

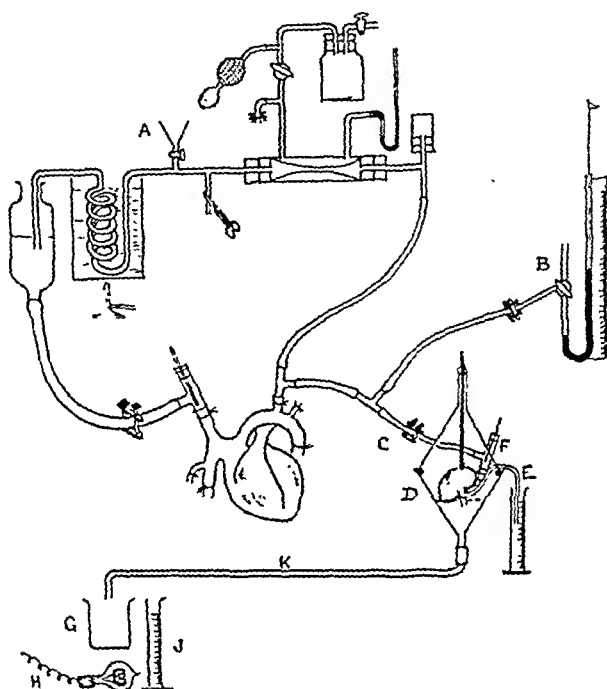


Fig. 1.

was taken to the heart-lung table and the cannula inserted into the slit in the aorta, pushed into the renal artery, and ligatured in place. The blood from the heart-lung was immediately turned on by opening the screw clip and the artificial perfusion thus started. The kidney was placed in a funnel, covered by another, and the whole surrounded by a cotton-wool jacket. A thermometer in the upper funnel gave the temperature of the chamber and another in the renal arterial cannula that of the blood supplied to the kidney. The blood from the renal vein was collected and returned periodically to the venous reservoir of the heart-lung preparation, the first few ounces being defibrinated before being returned. A mercurial manometer recorded the blood-pressure in the side tube leading to the kidney. The arrangement of the preparation when completed is shown in Fig. 1.

here was an interval of about 30 seconds during which there was

no blood supplied to the kidney The capacity of the ureteric cannula was 1 c c and urine began to flow at a time varying from five minutes to forty minutes after the artificial circulation had been established, the time tending to diminish as we acquired greater readiness in the insertion of the arterial cannula The amount of urine obtained varied according to the conditions of the experiment In some experiments the urinary flow attained the high value of 30 c c in ten minutes from a kidney weighing between 35 and 40 gms The urine thus obtained is not a mere transudate Its content in urea is always higher than that of the blood Thus in one experiment the relative amounts of urea in blood and urine respectively were

Urea mgms per cent		Rate of secretion c c in 10 mins
Blood	Urine	
45	561	3 8
33	256	3 0
31	93	6 2

The quicker the urinary flow the smaller was the percentage of urea But the absolute amount of urea excreted in a unit of time increased with increase in the urinary flow In the same way when glucose was injected into the blood, the percentage of this substance in the urine was always greater than that in the blood In one experiment the relative amounts of glucose in the blood and urine were

	Blood	Urine (total reducing substances)
Before adding glucose	07	079
	06	012
10 gms of glucose were then added to the circulating blood	78	2 54
	72	2 18

In this case there seemed to be a retention of glucose with small amounts in the blood, but a concentration of glucose in the urine when this substance exceeded the physiological limit in the blood

Influence of mechanical factors on the urinary secretion Richards(3) has recently described some experiments in which, by an ingenious method, he succeeded in maintaining the velocity of the blood through the kidney constant while varying the pressure at which it was forced through This was accomplished by inserting a pump in the course of the blood stream to the kidney of the rabbit without separating the organ from the rest of the animal The method necessitated the use of hirudin and will therefore be difficult to repeat until the manufacture of this substance is taken up again In our experiments the pressure at which the blood was supplied to the kidney was altered by altering the

artificial resistance of the heart-lung preparation and therewith the pressure on the arterial side of the system. Every alteration in arterial pressure naturally evoked an alteration in the rate of blood flow through the kidney, so that it was not possible to obtain so clean a dissociation between pressure and rate of flow as was the case in Richard's experiments.

In Fig. 2 we give the results of such an experiment. It will be noted that in most cases the velocity of blood through the kidney alters with the arterial pressure in the renal artery, but the alterations in the rate of flow are smaller percentually than the alterations in the blood-pressure. It will be seen that the urinary flow is proportional to the height of the arterial pressure but that there is a limiting pressure at which the urine ceases to be excreted even though there is a considerable

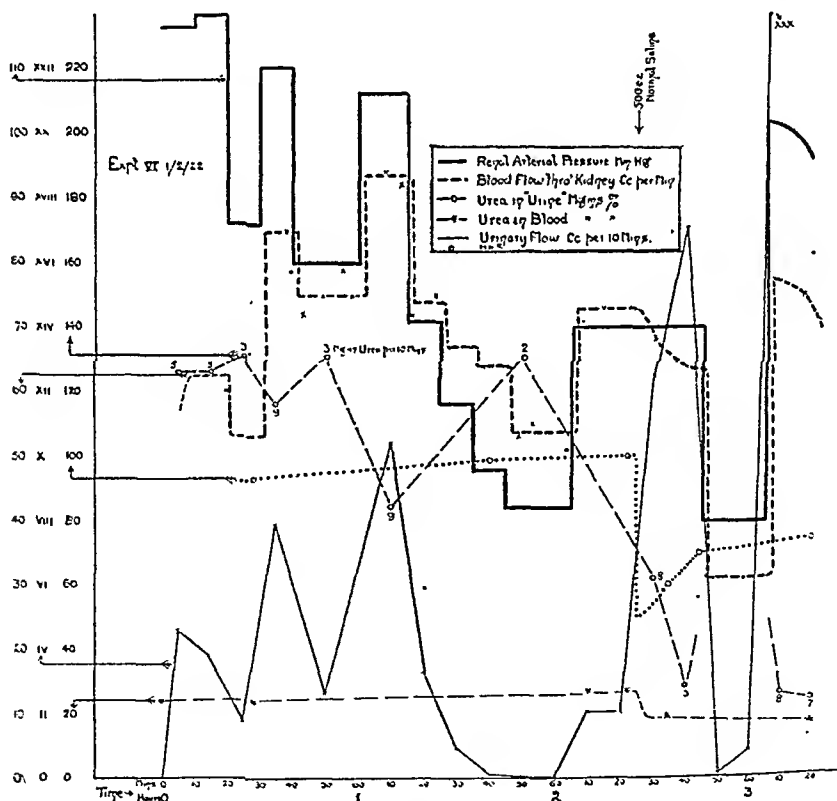


Fig. 2.

flow of blood through the kidney. Thus with a pressure of 106 mm. Hg. the urinary flow was at the rate of 12 c.c. in ten minutes, while the blood

flow, was at the rate of 187 c.c. per minute. On lowering the arterial pressure to 42 mm. Hg. the urinary flow ceased although the blood was still flowing through the kidney at the rate of 107 c.c. per minute. This is the same pressure at which the urinary flow ceases in the intact animal and corresponds, as was shown by one of us (4), to the osmotic pressure of the proteins of the blood *i.e.* about 30 mm. Hg. On diminishing the colloid contents of the blood serum by the addition to the blood of an equal volume of .9 % normal salt solution, the minimal pressure of secretion was diminished, so that at an arterial pressure of 40 mm. Hg. there was still a flow of urine at the rate of .4 c.c. per ten minutes.

It is interesting to note that in spite of the dilution of the blood in this case—a dilution which increased the urinary flow at a pressure of 70 mm. Hg. from 2 c.c. to 16.5 c.c. in the ten minutes—the velocity of the blood flow through the kidney actually fell off from 146 c.c. per minute to 128 c.c. per minute. The very great lowering in the colloidal concentration must have caused some swelling of the capillary wall or tissues of the kidney which impeded the flow of blood through this organ. In other experiments we found that addition of urea or of sodium sulphate to the circulating blood might cause an increased flow of urine without altering the rate of flow through the kidney. Judging from these few experiments we have therefore as the main factors determining the amount of urine secreted by the kidney (1) the blood-pressure in the kidney vessels, (2) the composition of the blood.

We propose to continue our experiments on the influence of changes in the composition of the blood on the urinary secretion. Before, however, we can continue this line of research we find that we must know more exactly the chemical changes in the blood under the conditions of the heart-lung preparation. In all our experiments the urine secreted by the perfused kidney has been hypotonic. Normally the molecular concentration of the urine is determined mainly by the urea and the sodium chloride. In our experiments we found that we could obtain a urine with a greater total molecular concentration than the plasma by the addition of urea or of sodium sulphate to the blood. In every case however the sodium chloride content of the urine was far below that of the blood plasma, and this in spite of the fact that a steady concentration of the plasma with its proteins and chlorides was going on in consequence partly of evaporation from the surface of the lungs, partly from the transudation of fluid into the lungs. Our attempts to account for this hypotonicity of the urine, *i.e.* the large retention of chlorides by the kidney, made us realise that the blood in the heart-lung preparation is

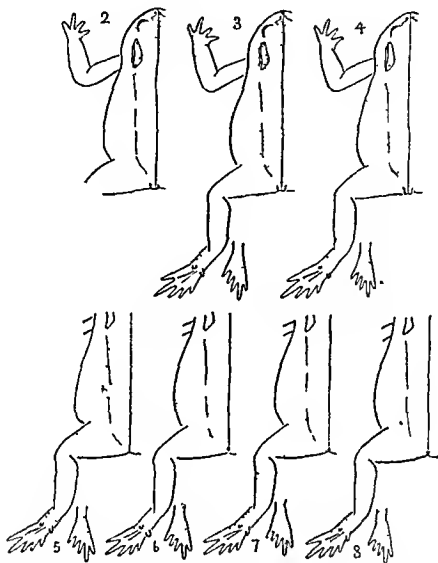
The 1st spinal root I found too short and delicate for isolation, but both Langley and Brücke state that the 1st spinal nerve does not send fibres to the sympathetic. Another delicate root, the 10th, was generally stimulated with the 9th and isolated in a few cases only. Experiments were made on twelve toads and as a rule all the spinal roots except the 1st were stimulated.

The roots can be distinguished with fair certainty by the muscular contraction they produce on stimulation. The 2nd, which of course can be told by its size, causes extension and adduction of the fore-limb. The 3rd to the 7th cause contraction in successive portions of the abdominal wall; the 3rd produces also slight flexion and pronation of the fore-arm, whilst the 7th causes adduction of the thigh. The 8th causes extension of the leg and flexion of the toes and the 9th extension of the leg and toes. The effect of stimulating the 8th and 9th depends on whether the arrangement of the nerves is anterior or posterior. The facts mentioned above refer to the general case; in the extreme posterior arrangement, weak stimuli applied to the 9th cause flexion of the toes while stronger stimuli cause extension.

The heart was not cut out, as it was in Brücke's experiments, since this abolishes the slight circulation that may remain after the operation. The clotting of blood in the spinal canal I found rather advantageous than otherwise, since the white roots floating in the red clot were easily seen.

Results. As in Brücke's experiments the amount of the secretion obtained on stimulating any given nerve root, and the extent of the area in which secretion occurred, varied considerably in different experiments, and to a degree outside any possible difference of anterior and posterior arrangement of nerves. There is no warrant for *thinking* that these differences are due to differences of innervation in different toads. They may I think in the main be attributed with certainty to differences in excitability caused by differences in the state of the circulation. It is known that the response given by pre-ganglionic fibres decreases with decrease of blood flow. By my method of operation the blood is slowed to an indeterminate extent, by Brücke's method the circulation ceases. The secretion obtained by stimulating nerve roots immediately after the operation described above is more copious, and the area affected more extensive than that obtained by stimulating it half-an-hour later and the later stimulation may have no effect. Recovery is slow in amphibia, and it is especially slow in absence of circulation; thus when nerves are stimulated in succession, stimulation of one tends to reduce the response of all others which supply the same glands. How far the glands are innervated by more than one spinal nerve I have not noted, but I have seen the same gland on the leg secrete on stimulating either

the 8th or the 9th nerve. It follows from these considerations that the most extensive area affected in any one experiment most nearly represents the normal innervation in all. In the figures I give the maximal



Maximal area of secretion obtained by stimulation of the 2nd to the 8th nerves
The dots represent roughly the amount of secretion

areas in which I have obtained secretion with each spinal nerve, how often they have been obtained I mention in the detailed account. I regard them as showing fairly accurately the normal innervation. Accurate results can probably only be obtained in toads with a normal circulation.

I may consider first the points which concern the general arrangement of the pre-ganglionic sympathetic fibres. The most fundamental is the origin of sympathetic fibres from the nerves giving origin to the pelvic nerve (sacral autonomic). According to Langley and Orbell, the pelvic nerve arises from the 9th and 10th nerves and these containing no sympathetic pre-ganglionic fibres, they found (3) that section

hind-limb. In no case was the secretion greater than with the 7th; in three cases it was much less, and in two slightly less than with the 7th. In each of the five experiments the area of secretion was nearly the same as with the 7th.

Variation in lower spinal nerves. As was pointed out by Langley the variation in the arrangement of the lumbar plexus is not marked in toads. I have paid attention to the size of spinal roots and nerves in several toads especially when the 8th root was effective. In the 7th to the 9th nerves the posterior root is usually larger than the anterior root, the 8th and 9th being nearly of the same size and much the same in different animals though there is slight variation. On the other hand the size of the 8th and the 9th anterior roots varies greatly. In extreme cases either the 8th or the 9th can be twice as large as the other and in such cases it can often be followed in two bundles to the spinal ganglion. Slight variation in the size of the anterior roots does not always reveal itself in the lumbar plexus, sometimes the variation is the reverse of that in the anterior roots. Still there seems to exist some relation between the size of the anterior roots and the presence of the sympathetic fibres in the 8th root. In three out of five toads in which secretory fibres were found in the 8th root, the 9th anterior was twice as large as that of the 8th, in one the 9th nerve was larger than the 8th. Though this suggests the posterior arrangement of the anterior roots in the lumbar plexus, there was still one case in which the 8th anterior root was effective and slightly larger than the 9th anterior root. The 7th anterior root looked rather smaller than usual in these cases but no accurate estimation was made. In four other cases in which the 8th root had no effect the 8th anterior root was either much larger than the 9th and in two bundles, or of nearly the same size.

I conclude that variation occurs chiefly in the 8th and 9th anterior roots, not necessarily including the 7th anterior root, and that the presence of the secretory fibres in the 8th root is often associated with the extreme cases of the posterior arrangement though in other cases shifting down of some fibres from the 7th may occasionally occur.

Stimulation of the sympathetic trunk. Another method of determining the origin of the sympathetic fibres is to stimulate the sympathetic trunk. This I have done from above downwards and from below upwards. In these experiments, either urethane was given and the brain destroyed, or the brain and cord destroyed without giving urethane in order to avoid the chance of reflex secretion, but no difference in result was found by the two methods. The sympathetic trunk was exposed,

a spinal nerve was tied, cut peripherally of the ligature and at its exit from the vertebra. The sympathetic was cut and isolated up to the next ganglion. The spinal nerve was then held up and the sympathetic trunk immediately above or below it was stimulated. The spinal nerves and the sympathetic trunk were successively treated in the same way.

In five toads the sympathetic was thus stimulated from above downwards beginning between the 3rd and 4th ganglions. The main object of these experiments was to see how far up the chain stimulation would cause secretion in the hind-limb. In two experiments out of five, stimulation above the 4th ganglion caused secretion in the whole extent of the leg and in the foot. In two experiments out of four secretion in the same area was obtained by stimulating above the 5th ganglion. The relative amount of secretion in the thigh and lower leg varied in the different experiments. In the back the secretion began in the region of the peripheral distribution of the nerve immediately below the point stimulated. Stimulation at lower levels caused secretion in the whole of the dorsal surface of the limb in all cases, but the secretion varied considerably in amount.

In the series in which the sympathetic was stimulated from below upwards the object was to determine the lowest part capable of causing secretion in the fore-limb and head. Experiments were made on four toads, beginning the stimulation below the 5th ganglion. The lowest part from which secretion in the head was obtained was below the 4th ganglion. It was obtained in one case only and it was a slight secretion in one side of the head. Stimulation below the 3rd ganglion in two cases caused secretion in the lower half of the head and some in the fore-limb. Stimulation below the 2nd ganglion caused copious secretion in both head and fore-limb. In one case there was a good secretion on the skin of the lower jaw and in the skin of the flexor side of the fore-limb.

The range of innervation in these experiments conforms closely to that found in those in which the spinal nerves inside the vertebral canal were stimulated. But in stimulating the sympathetic trunk there is some risk of the current spreading to the pre-ganglionic fibres of the neighbouring root. Whilst I do not think this occurred in my experiments I have no objective evidence of this to offer.

Finally I may mention that I have stimulated the peripheral ends of the dorsal cutaneous nerves of the trunk and find that there is little or no overlapping of their secretory area, *i.e.* of the areas of the post-ganglionic fibres running to the trunk by the successive spinal nerves.

ment of the zero for a very long time after setting up the muscle (three hours or more), which cannot be due to the ordinary process of settling down to uniformity of temperature within the chamber, nor to any external magnetic disturbance affecting the galvanometer; as this slow movement of the zero is invariably in the same direction, viz. opposite to that of a deflection corresponding to a production of heat, it may be due to some process of evaporation. From the experimental point of view we could not always wait till this movement became of negligible amount, but it was always measured for two or three minutes just before each record, and if it was not negligible a correction was made by assuming that the zero moved in the same way during, as it did just before, the record. The correction introduced in this way was always small. It is of course possible that, after a stimulus, there may be a considerable evaporation (e.g. of CO_2) from the muscle before there is approximate equilibrium in the chamber again; in fact, we have frequently observed that this progressive movement of the zero is greater, after giving a few preliminary stimuli, than before. In this case our numerical results, especially for the heat-production soon after the stimulus, will be very slightly too small.

Apart from this motion of the zero it was necessary to wait for at least $1\frac{1}{2}$ hours, after setting up the muscle in the chamber, before a sufficiently reliable record could be taken; at least an hour after changing or removing a solution, and at least half-an-hour after changing the gas in the chamber. Further, it is not possible to take reliable records at intervals of less than a quarter of an hour at high temperatures ($20^\circ\text{C}.$), or of less than half-an-hour at low temperatures ($0^\circ\text{C}.$). It was attempted at first to carry out observations at $0^\circ\text{C}.$ as it is easier to keep the temperature in the flask outside the chamber very uniform, and the muscle usually remains in good condition for a long time. It was found, however, that at low temperatures the recovery process goes on so slowly, that although the rate of heat-production can be fairly well observed for about ten minutes after the stimulus, no accuracy is possible in the measurement of the *total* heat of the recovery process. The most suitable temperature was found to be $20^\circ\text{C}.$, at which the recovery heat in oxygen, for a long tetanus (0.5 sec.), has reached in six minutes some 95 p.c. of its total value. At still higher temperatures the muscle is apt to deteriorate. A few experiments have been carried out also at $15^\circ\text{C}.$ and at $0^\circ\text{C}.$ All contractions were rigidly isometric.

Photographic recording of the galvanometer deflection was employed, and the analysis of the records was carried out by the method previously

described (1) but employing an interval¹ of 5 or 10 secs., instead of $\frac{1}{4}$ sec. or less, as used for the analysis of the initial heat. The method is somewhat laborious, but it is fairly accurate, and the results are consistent.

The experiments in oxygen require no description. Those without oxygen were made in nitrogen which, in the later experiments, was passed in very small bubbles through two bottles of alkaline pyrogallol to ensure its complete freedom from oxygen; in the earlier experiments the nitrogen was taken directly from a cylinder, but there does not seem to be any appreciable difference due to the further treatment of the nitrogen. In the nitrogen experiments the muscle was first soaked, in the chamber, in Ringer's solution which had been recently boiled to free it from oxygen, and the chamber and connecting pipes were carefully left quite full of this solution before it was blown out with the nitrogen. In some experiments the absence of oxidations was further ensured by the use of potassium cyanide. In the KCN experiments the muscle was soaked, inside the chamber, in KCN-Ringer solution of the strength, and for the time necessary. The solution was then blown out with nitrogen.

The recovery heat-production has been expressed here in terms of the initial heat-production, i.e. in terms of the total heat set free in the three initial anaerobic phases (4). Thus a total recovery heat of 1.5 means a recovery heat equal to 1.5 times the total initial heat, while a rate of recovery heat-production of .004 per sec means .004 times the total initial heat per sec. In the diagrams will be found tables showing the initial heat in absolute units (cals. per gram) for different times of tetanus, so that the absolute rate of recovery heat-production can be calculated if required. It will be seen that as small a rate as 10^{-6} cal. per gram per sec. has sometimes to be observed, the records frequently extending far beyond the first five minutes shown in the diagrams.

It is not usually advisable to rely too far on the record and analysis for more than five or six minutes after the stimulus, and (as seen in Fig. 1) the recovery heat-production is by no means complete at that stage. The falling curve however has generally become reasonably ex-

¹ It was found, after several of the analyses had been completed, that an error had arisen due to taking the interval used in the analysis appreciably greater than the time taken by the galvanometer to reach its maximum deflection. This had the effect of making the calculated heat too large, but it does not alter the shape of the curve showing the rate of heat production. The error (never more than 10 p.c.) was found by analysing, with a shorter interval, some of the experiments which had been analysed with the longer interval, and corrections have been made in all experiments in which the longer interval was used.

ponential in character by then, and the remainder of its area can be simply calculated from the ratio of its successive ordinates at equal

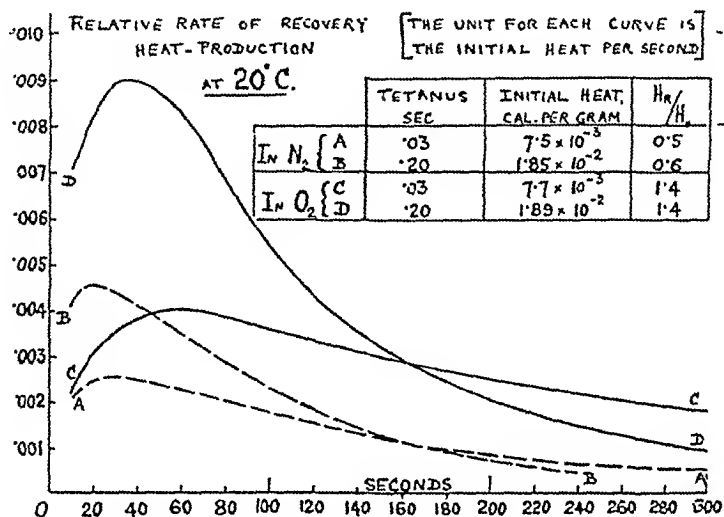


Fig. 1. Delayed heat-production in presence and absence of oxygen, medium and short tetanus.

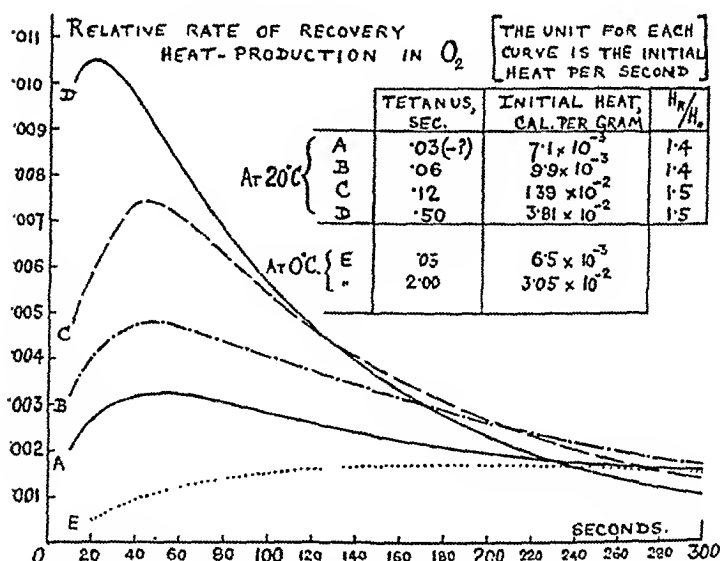


Fig. 2. Recovery heat-production in oxygen: four times of tetanus at 20°C.; two times of tetanus at 0°C. (results coincide).

intervals of time, and allowed for as a correction. The *total* recovery heat-production has always been corrected in this way. In cases of longer

stimuli, *e g* as shown in curve *D*, Fig 1, no appreciable error can result from this, the calculated correction, after six mins at 20° C, being only 6 p c of the whole in cases of very short stimuli, where the curve comes down less steeply (*e g* curve *C*, Fig 1), the calculated correction after six mins is greater, viz up to 30 p c of the whole, and the final result correspondingly less reliable. On the whole, however, we should say that all our values of the total recovery heat production are correct within 10 p c, most of them within much less.

It will be noticed that the diagrams of heat rate are not drawn for the first 10 seconds. The analysis is complicated by the fact that, especially with a longer tetanus, all the "initial" heat does not occur quite instantaneously at the start.

As the results given in this paper are based on observations and analysis requiring all the accuracy possible, it may be remarked that in no case has a single photographic record been employed, two have always been taken and if they did not agree closely then the mean of three for "controls" (by artificial heating) the mean of never less than three records has been employed in the analysis. More than 50 experiments on separate frogs have been made, and over 100 complete analyses of records carried out.

Results Typical results are shown in Figs 1, 2 and 3. Consider curve *D*, Fig 1, corresponding to a 0.20 sec tetanus in oxygen, giving rise to an initial heat production of 1.89×10^{-2} cal per gram. The rate of recovery heat-production starts at a low level, rises rapidly, attains a maximum about 35 secs after the stimulus and falls slowly towards zero. The total recovery heat is 1.1 times the initial heat. The general

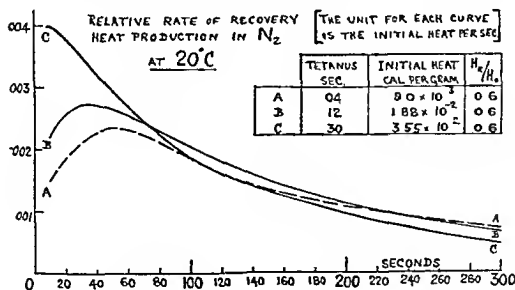


Fig 3 Delayed heat production in nitrogen, three times of tetanus

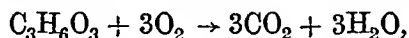
form of this curve is invariable, and the total recovery heat in oxygen (for various times of tetanus) had the following values (expressed as

multiples of the initial heat-production) in 20 observations, in 14 separate experiments, at 20°:

1.2, 1.3, 1.3, 1.4, 1.4, 1.4, 1.4, 1.4, 1.4, 1.4,
1.4, 1.5, 1.5, 1.5, 1.5, 1.5, 1.5, 1.65, 1.8, 1.8,

the mean being nearly 1.5; we will consider the consequences of this later.

The most striking and (to us) unexpected characteristic of the curve is its rise to a maximum. We had expected the chemical processes associated with oxidative recovery to start at a high level, and to decline continually to zero: the only explanation we can offer of the rise to the maximum is that these chemical processes occur in several stages, and that the main portion of the heat is liberated in one of the later stages rather than in the first. Suppose, for example, that lactic acid is oxidised during the recovery process. Then, if it were oxidised directly in one stage,



the rate of heat-production should be greatest at first (when the concentrations of $\text{C}_3\text{H}_6\text{O}_3$ and of O_2 are greatest), and should fall continually, as the reaction goes on, according to the laws of mass action. If however the reaction occurred in two stages, for example,



the second (oxidative) stage represented by equation (II) being responsible for the larger part of the heat, then the rate of heat-production (depending chiefly on the progress of the oxidative stage of equation (II)) would be low at first, would rise as the anaerobic stage of equation (I) proceeded and provided equation (II) with readily oxidisable material X , would reach a maximum, and fall again to zero as observed. This hypothesis is strengthened by the fact that some heat is given out, even in the complete absence of oxygen: it is certain that some delayed anaerobic reactions do occur.

Consider next Fig. 1, curve C , corresponding to .03 sec. tetanus in oxygen, and to an absolute initial heat-production of 7.7×10^{-3} cal. per gram. The curve is of the same type as before, and the total recovery heat is practically the same fraction (1.4) of the initial heat: we notice however that the recovery process is carried out relatively more slowly than in the more powerful contraction. It should be noted particularly that Figs. 1 to 3 give only *relative* values: we had expected both the absolute rate at any given moment, and the total absolute value of the recovery heat-production, to be proportional to the magnitude of the

initial heat-production, in which case the *relative* curves of Figs. 1 to 3 would all have been the same for the different initial breakdowns caused by different durations of stimulus. All our experiments tend to verify the second item, viz. that the *total* absolute value of the recovery heat-production is proportional to the *total* initial breakdown preceding it. With equal consistency however they show that the curve of recovery rises and falls relatively more steeply in the case of the more energetic response. Examining curves *D* and *C* more closely it will be seen that, during the first half minute, the relative rate of heat-production is about $2\frac{1}{2}$ times as great in *D* as it is in *C*, and the initial heat-productions are also approximately in the ratio $2\frac{1}{2} : 1$; thus the absolute rate for the first half minute is $(2\frac{1}{2})^2$ times as great, but this ratio of the absolute rates falls off continually, the curve *D* descending faster than *C*, until they may become almost equal at some time beyond the five minutes shown on the diagram. Now the initial heat-production is a measure of the concentration of the bodies whose removal constitutes the recovery process: in this case, therefore, the recovery process appears to go on at a rate proportional to the *square* of the concentration of the reacting bodies, a fact which can be interpreted by supposing the rate of the recovery process to be governed by some *bimolecular reaction*. What this bimolecular reaction may be we can only guess: its existence however may afford some clue, in the further exploration of carbohydrate breakdown and reconstruction in muscle. The same result is shown by other observations. For example, in the experiment the results of which are shown in Fig. 2, the initial heat production (H_0) was varied by varying the duration of the stimulus, and the recovery heat-production (H) during the first half minute was estimated. It is seen that (comparing only the numbers in the same experiment) H/H_0 is by no means constant, but H/H_0^2 is not far from constant, from which we may conclude that, at any rate in its early stages, the recovery process is governed by some bimolecular reaction.

Tetanus: sec.	.03	.06	.12	.5
Initial heat-production, H_0	7.1×10^{-3}	9.9×10^{-3}	1.39×10^{-2}	3.81×10^{-2}
Recovery heat-production in first half min., H	4.9×10^{-4}	9.8×10^{-4}	2.1×10^{-3}	1.02×10^{-2}
H/H_0	.07	.10	.15	.27
H/H_0^2	10	10	11	7

All the experiments discussed above were with muscles apparently in good condition at the end of the experiment, as judged by the uniformity of the initial heat-production to successive maximal stimuli. When (as judged in a similar manner) the muscle was failing, quite abnormally large values were obtained for the recovery heat, up to 5.5

in the extreme case observed. One of the signs of failure is great wastefulness in recovery. This fact is of importance in a comparison of our heat measurements with the chemical determinations of Meyerhof, and will be further discussed below.

Consider now Fig. 1, curves *A* and *B*, and Fig. 3, made in the complete absence of oxygen. In A. V. Hill's original investigation (6) it was shown that the delayed heat-production in the absence of oxygen is much smaller than in its presence, and it was assumed that if the oxygen were completely eliminated there would be no delayed heat: as a matter of fact, examining the earlier records now, there are obvious signs of a delayed heat-production in nitrogen, in amount however which the method employed then was incapable of measuring with any accuracy. There is no doubt now however either of the existence, or of the magnitude, or of the time-relations, of the delayed heat-production occurring in as complete an absence of oxygen as it is possible to attain. Curves *A* and *B*, Fig. 1, are typical of many experiments performed, and it is found that the curves are of the same general shape as those in oxygen and are similarly affected by the duration of the stimulus; but there is an invariable difference in the shapes of the curves in nitrogen and in oxygen, in that the former always rise more quickly to and descend more quickly from their maxima (for the same time of tetanus), with the consequence that, although the maximum rate of delayed heat-production when in nitrogen is always about one-half the maximum rate when in oxygen (for the same time of tetanus), the area of the whole curve when in nitrogen, i.e. the total delayed heat-production, is always about one-third of that when in oxygen. Fig. 4 has been drawn to show this numerically, at 20° C., for times of tetanus from 0.03 to 0.5 second; it contains the average results of 14 experiments in oxygen and 20 experiments in nitrogen, 8 of these being for the same muscle in oxygen and nitrogen; these results were in good agreement and there seems to be no doubt about the difference described, which points to the conclusion that the delayed heat-production in nitrogen is due to a different process from that in oxygen. Further, it may be observed that, if the total delayed heat-production when in oxygen is the sum of two parts (1) the delayed heat-production when in nitrogen, (2) the recovery heat due to oxidative processes alone, then the latter will differ from the curves in nitrogen even more than do the curves here shown in oxygen; in fact, the delayed heat-production in nitrogen is about one-half the heat-production due to oxidative processes alone, but it must occur relatively at about twice the rate.

The total delayed heat-production in nitrogen, for various times of tetanus, had the following values, expressed as a fraction of the initial

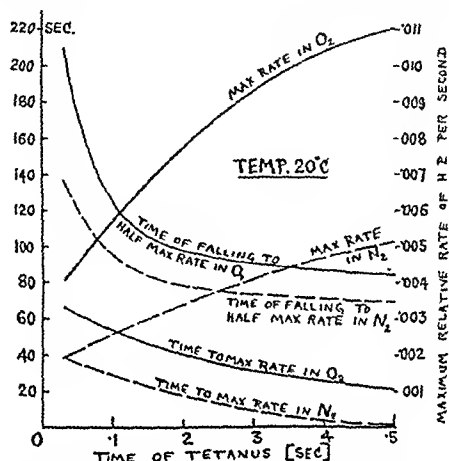


Fig. 4. To illustrate the difference between the curves of delayed heat production in oxygen and in nitrogen, and their dependence on the duration of the stimulus. The maximum relative rate of delayed heat production is reached more rapidly in nitrogen, and more rapidly with the longer tetanus. The relative rate at which the curve falls from its maximum is greater in nitrogen, and greater with the longer tetanus.

heat-production, in 29 observations, in 20 separate experiments, all at 20° C.:

0.3, 0.3, 0.3, 0.35, 0.4, 0.4, 0.4, 0.4, 0.4, 0.45, 0.45, 0.5, 0.5, 0.5, 0.5,
0.5, 0.55, 0.55, 0.55, 0.55, 0.6, 0.6, 0.6, 0.6, 0.6, 0.6, 0.6, 0.65, 0.65,

the mean value being very nearly 0.5¹. Now it seemed possible, though very improbable seeming what precautions had been taken to exclude oxygen, that this small delayed heat-production (one-third of that in oxygen) was really due to oxygen still present in the muscle. In order finally to eliminate this possibility experiments were undertaken in which the muscle was not only in nitrogen, but had previously been soaked in a KCN solution. It is known—see *eg* Weizsaecker (13)—that a very dilute solution of KCN practically eliminates the possibility

¹ The mean in four experiments at 15° C. was practically the same.

of oxidation. It is not desirable to expose the muscles either too long, or to too great a concentration of KCN, since they have to remain on the thermopile at 20° C. for at least an hour in N₂ after treatment in order to secure a stable zero, and then to give a series of good and consistent readings: any kind of injurious treatment is apt to leave the muscle in a failing condition before the series is complete. It was found possible however, after an experiment of the usual kind in N₂, and without removing the muscle, to replace the N₂ by oxygen-free Ringer's solution containing KCN in strength about .001*n*, to expose the muscles for about a minute to this, and finally to replace the solution by nitrogen. Many experiments made in this way showed a failing muscle, and a consequent increase in the delayed heat. In each of the following however the muscle was in excellent condition at the end of the experiment.

Experiment	Tetanus, sec.	Delayed heat
1. In N ₂	.03	.40
" "	.20	.45
After KCN .0007 <i>n</i> for 1 min.	.03	.35*
" " "	.20	.30
2. In N ₂	.03	.5
" "	.30	.6
After KCN .001 <i>n</i> for 1½ min.	.03	.2*
" " "	.30	.3

After treatment with KCN the maximum rate of delayed heat-production was nearly always less than the corresponding amount in N₂ before treatment, and for the shorter tetanus especially, the rate fell off more slowly than in N₂ with the consequence that the estimates of the total delayed heat in certain cases (marked * above) cannot be made with great accuracy. However, from these and from other experiments of less value, it is clear that the presence of KCN has reduced the delayed heat-production from an average value of about 0.5 to an average of about 0.3. Thus the most drastic treatment still leaves a delayed heat-production of about one-third of that occurring in the initial processes of contraction. We believe this to be a genuine effect, and not due to oxygen still remaining in the muscle—it must be remembered too that after several hours anaerobic survival at 20° C., appreciable quantities of lactic acid will be present: any available oxygen will have been used long since. There seems therefore no escape from the conclusion that in a contraction after several hours anaerobic survival at 20° C. there is a delayed heat-production amounting to some 50 p.c. of the initial heat-production: that this is not (at any rate entirely) abolished

by KCN: and therefore that it is of a non-oxidative character. The time-relations of this anaerobic delayed heat-production are rather different, as is shown in Fig. 4, from those of the oxidative delayed heat-production. It may be that it corresponds to preliminary non-oxidative stages of the recovery process, or to the prolongation of the normal chemical processes accompanying relaxation.

Bernstein⁽¹⁾ expressed some doubt of the adequacy of A. V. Hill's original method⁽⁶⁾ to provide an accurate measure of the total heat: admittedly the results given by it were to some degree a matter of estimation: the experiments now described, however, are probably more accurate than the muscles are consistent, and the variations found can scarcely be attributed to the method, but rather to differences between, and changes in, the muscles used. On the average, in the sartorius muscle of *Rana temp.*, in good condition in oxygen, the total delayed heat cannot be far from 1.5 times the initial heat.

The amount of lactic acid produced in a contraction can be calculated from the heat-production, if we know the amount of heat accompanying the anaerobic formation of 1 gram. of lactic acid. The original determinations of this quantity were made by A. V. Hill⁽⁵⁾, then followed the experiments of Peters⁽¹¹⁾, and finally the very careful and accurate series of observations by Meyerhof^{(7), (10)}. In his last paper, of which we have been enabled by his kindness to see the MS, Meyerhof concludes that 370 cal. are liberated in the anaerobic production in muscle of 1 gram. of lactic acid: we will assume this quantity as the basis of our subsequent calculations. We may prepare then the following "balancesheet"; in doing so we have assumed a rather low relative value of the total delayed anaerobic heat-production, viz. 0.3: this is less than the mean value found above, though not less than several individual values: any error however would probably tend to make it too high, and the fact that it is rather less after treatment of the muscle with KCN suggests that possibly some small residual oxidations are leading to a small positive error, in spite of all the precautions we have taken.

Heat production in the liberation and removal of 1 gram. of lactic acid in a muscle in oxygen.

Phase	Relative value	Absolute value, calories
Initial anaerobic	1.0	285
Delayed anaerobic	0.3	85
Total anaerobic	1.3	370
Total delayed heat	1.5	425
Delayed anaerobic	0.3	85
Difference, delayed oxidative	1.2	340

Let us suppose that of the 1 gm. of lactic acid we are considering x gm. is oxidised in recovery, and $(1 - x)$ gm. is restored to its previous state (as glycogen; Meyerhof(9)). The muscle is finally exactly as it started, except that the glycogen equivalent of x grms. of lactic acid has disappeared: the heat of combustion of glycogen, according to Stohmann(12), is 4191 cal. per gm.: according to Emery and Benedict(2) it is 4227 cal. per gm.: we will assume the mean of these quantities, viz. 4209 cal.: hence the heat of combustion of 0.9 gm. of glycogen, the amount corresponding to 1.0 gm. of lactic acid, is 3788 cal. Hence the total energy available to cover all breakdowns in the complete cycle is $3788 \times x$ cal. Equating this to $(285 \div 85 \div 340) = 710$ cal. we find $x = 710/3788 = 0.188$. Thus of 1 gm. of lactic acid passing through the complete chemical cycle of contraction and recovery, 0.188 gm. is oxidised, and the remainder, viz. 0.812 gm., is restored to its previous state as glycogen. Thus only from one-fifth to one-sixth of the lactic acid is oxidised—the remainder is “restored.” Meyerhof, working on muscles stimulated to fatigue and recovering slowly in oxygen, has found one-third to one-fourth of the total lactic acid to be oxidised(8), (9). It is unlikely that the difference between his result and ours is due simply to an error of observation on either side, and he has suggested to us privately that the difference may be due to the fact that his methods necessitated the use of muscles which had been stimulated for some time and fatigued, and in which—owing to their bulk—the oxygen supply was relatively so inadequate that the recovery occupied a very long time: we, on the other hand, have been able to employ a single stimulus only, and a very liberal supply of oxygen. This suggestion is supported by our observation that a failing muscle in oxygen gives quite abnormally large values of the delayed heat-production, and presumably therefore of the proportion of lactic acid oxidised. We may conclude therefore that, in the oxidative recovery of fresh muscle, only one lactic acid molecule in five or six is oxidised, the remainder being restored (as Meyerhof has shown) as glycogen.

In his recent paper Meyerhof has shown that the heat of combustion of concentrated (70 p.c.) lactic acid to CO_2 (gas) and water (fluid) is 3615 cal. This agrees exactly with Emery and Benedict's(2) determination on (about) 90 p.c. lactic acid. Meyerhof calculates that the heat of 111-fold dilution of 70 p.c. lactic acid is 14 cal. per gm., and of 10 p.c. acid 18.5 cal. per gm.: assuming the heat of dilution of 90 p.c. acid to be 17 cal. per gm., Emery and Benedict's figure for the heat

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The effect of temperature. No exact quantitative expression of the effect of temperature on the recovery process has been found possible. There is no doubt, however, that a fall of temperature very considerably prolongs the anaerobic, as well as the oxidative, processes of recovery, and reduces their maximum rate: there is no evidence however that it changes the total extent of the delayed heat-production. In general we feel justified in stating that the effect of temperature on the velocity of the recovery or delayed anaerobic processes, in the presence of excess of oxygen, or in its absence, is of the same order of size as one expects to find for other chemical reactions occurring in the living organism. This shows that the rate of recovery is not dependent on a physical process, such as the diffusion of oxygen into the muscle: as a matter of fact a muscle saturated with oxygen, even at 20° C., should contain about .025 c.c. per grm., an amount sufficient to raise the temperature by about $\frac{1}{8}$ ° C. if used in the combustion of sugar: this is far greater than the effect of any stimulus we have applied, so that dissolved oxygen was always present in sufficient quantity to carry out recovery without further diffusion. The slow rate of recovery at a low temperature is shown in Fig. 2. This slow rate is of particular interest in relation to the activity of cold-blooded animals: in such animals, at a low temperature, recovery must be so slow that no considerable or prolonged exertion could be undertaken without producing prolonged fatigue.

SUMMARY.

A new investigation has been completed of the course and magnitude of the heat-production associated with the recovery phase of muscular contraction.

(1) There is a delayed production of heat after activity, both in the presence and in the absence of oxygen, though much larger in the former case. This heat-production starts at a low level, rises to a maximum, and then falls slowly to zero, the whole process occupying about ten minutes at 20° C. and longer at lower temperatures. The effect of temperature shows that the velocity of the process is controlled by that of some chemical reaction. The shape of the curve, and the position of its maximum, but not its total area, depend upon the extent of the initial breakdowns.

(2) The shapes of the curves show (a) that the anaerobic and the oxidative processes are of different characters: (b) that the recovery process takes place in at least two stages; and (c) that its rate is governed by that of some unknown bi-molecular chemical reaction.

(3) The mean value of the total oxidative recovery heat-production is 1.5 times the total initial heat-production; the mean value of the total delayed heat, in as rigorous an absence of oxygen as it is possible to attain, is 0.5 times the initial heat-production; treatment with KCN somewhat diminishes, but does not abolish, this delayed anaerobic heat.

(4) It is concluded that in the oxidative removal of lactic acid, from one-fifth to one-sixth of the lactic acid is burnt, the remainder being restored as glycogen. The difference between this value, and the one-third to one-fourth of Meyerhof, may be due to the better condition of the muscles in our experiments: in a failing muscle recovery is more wasteful.

(5) The total initial heat-production is about 285 cals per gram of lactic acid set free. This corresponds to the heat evolved in the production of lactic acid from glycogen, and its neutralisation chiefly by the alkaline protein buffers of the muscle (Meyerhof). It is concluded that these chemical processes are the only ones of importance occurring in the initial phases of contraction, the production of acid leading to a rise of tension, its neutralisation to relaxation.

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THE NERVE FIBRE CONSTITUTION OF PERIPHERAL NERVES AND OF NERVE ROOTS. BY J. N. LANGLEY.

(From the Physiological Laboratory, Cambridge.)

IN the following pages I give an account of several points relating to the constitution of nerves and nerve roots which have either been little investigated, or which have given rise to difference of opinion.

One important point is whether non-myelinated fibres are present in small or large numbers in the posterior roots. Ranson has shown in a series of papers that the silver impregnation method brings out in spinal nerves(1) and in their posterior roots(2) deeply stained small fibres in numbers greater than that of the myelinated fibres. These he considers to be non-myelinated fibres arising—so far as they are not of sympathetic origin—from small cells in the spinal ganglia and to be afferent fibres. Starting from these premises he concludes, as the result of other observations, that non-myelinated afferent fibres are a special class, the function of which is to cause a rise of blood-pressure and to give rise to “protopathic” sensation(3). When Dr Nakamura was working in the Cambridge Physiological Laboratory in 1920 I asked him to investigate the nature of the fibres described by Ranson. He compared sections of the sciatic nerve (rabbit and cat) treated by osmic acid, and by the Ranson method. In the nerves treated with osmic acid he found many more small myelinated fibres than were obvious in the nerves treated with silver nitrate. This suggested that some of Ranson’s fibres were small myelinated fibres. At this stage Dr Nakamura’s stay in this country ended.

Method. I have confined my observations to nerves treated with osmic acid, since this treatment gives decisive results on the questions I wished to examine. The animals, chiefly cats, were bled to death whilst chloroformed. The nerves were placed in 1 p.c. osmic acid for 16 to 24 hours, and washed for some hours in running water. They were sometimes taken the day after death (the body being kept cool in the interval) and apparently without disadvantage. The myelin takes a darker tint if the nerves are treated with 12 p.c. formaline for a quarter to half an hour before osmic acid, or if they are placed in a mixture of formaline and osmic acid, but good preservation was then less constant. A portion

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fibres are obvious and around them is a considerable amount of fine connective tissue. The non-myelinated fibres are relatively more numerous in the dorsal cutaneous branches than in the other cutaneous nerves, and with the connective tissue take up about half of the area of the cross section. They appear also to be somewhat more numerous in the internal saphenous nerve of the rabbit than in that of the cat.

The nerves to skeletal muscle examined were those to the gastrocnemius, tibialis anticus, and extensor comm. digitorum. In these the brown stained tissue occurs in small patches only. It varies in amount in the several branches of the nerves, some have a few such patches, some have one or two only; they never take up more than a very small part of the transverse section of any of the larger branches. As the nerves divide into small branches near the muscle, the brown stained tissue is apt to be still more unequally distributed, some minute branches have relatively much, others none. On teasing out the nerves, the brown stained tissue is seen to consist largely of non-myelinated fibres as it does in the cutaneous nerves.

The difference between cutaneous and muscle nerves is shown in Pl. I, figs. 1 to 3. Fig. 1 is a bundle of a dorsal cutaneous nerve in which most of the non-myelinated fibres were stained throughout and consequently appear as solid masses. Fig. 2 is a bundle of a ventral cutaneous nerve in which the non-myelinated fibres were slightly swollen and appear as rings. Fig. 3 is part of a bundle of the nerve to the inner head of the gastrocnemius muscle; in the whole bundle there were only three small patches of non-myelinated fibres; these are indistinctly shown in the figure, one is at the edge and contains three small myelinated fibres. The non-myelinated fibres, however, are not always quite so few as in this specimen. But it is clear that many non-myelinated fibres run to the skin and a few only to the muscles.

The nerves which run both to the skin and to skeletal muscle have intermediate characters. The intercostal nerves near the cartilage, where they are mainly muscle nerves, differ but little from a muscle nerve, the posterior tibial has a small portion almost entirely resembling a muscle nerve, the rest is more like a cutaneous nerve, but has more fibres 2 to 3.5 μ in diameter. The musculo-cutaneous is mainly like a cutaneous nerve but the non-myelinated fibres are more unequally distributed.

Nerve trunks. *Rauvier*(5), in describing the components of nerve trunks, said that the non-myelinated fibres occupied a scanty part (*une faible partie*) of the cross section. Broadly speaking, this is true, but the statement suggests much fewer fibres than are in fact present, and it

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Nerve trunks. Rauvier(5), in describing the components of nerve trunks, said that the non-myelinated fibres occupied a scanty part (*une faible partie*) of the cross section. Broadly speaking, this is true, but the statement suggests much fewer fibres than are in fact present, and it

thin, feebly staining membrane from the arachnoid surrounds each rootlet, but sends only the most delicate strands of tissue between the nerve fibres. Usually all that is to be seen between them is here and there a capillary or a small vein. Figs. 4 and 6, Pl. I, are from the posterior root of the 6th lumbar nerve, and Fig. 5 from that of the 7th cervical. The fibres are more separated in the latter and thus show better the complete absence of non-myelinated fibres: the absence was still more distinct under the microscope. The nerve roots after treatment with osmic acid are, as is known, difficult to tease since they are apt to break transversely but with patience preparations can be made in which every fibre is visible. I have teased out two-thirds of the 13th thoracic, 4th lumbar and 2nd sacral posterior roots, the whole of the posterior roots of the 3rd sacral nerve, and portions comprising several hundred myelinated fibres of the lower cervical and lower lumbar nerves, and have never found in any one of these preparations more than half a dozen fibres which had any resemblance to non-myelinated fibres. I am very doubtful whether any enter the spinal cord with the roots. The state is much the same in the anterior roots, but I have not made any exact comparisons between the two roots as regards the non-myelinated fibres.

General points regarding the myelinated fibres. In transverse sections the two roots can be distinguished though with varying ease. The anterior roots contain relatively more large nerve fibres of approximately the same size. The posterior roots, though containing large nerve fibres, consist of fibres of different size in more equal proportions. The distinction is best seen with a magnification sufficiently low to bring a large part of the root into the field of the microscope. It is very obvious in the lower cervical and lower lumbar roots (cp. Figs. 3 and 4 in the text), and relatively slight from the 2nd sacral roots downwards; it is shown in the microphotographs of the roots of the 1st coccygeal nerve given by Dale(s). The difference is due to the fibres of about 7.5 to 11 μ in diameter being relatively and absolutely fewer in the anterior roots than in the posterior roots; the size varies slightly in different nerves.

The different rootlets of a root frequently vary in the relative number of fibres of different size which they contain. One rootlet may have a considerable number of the smaller fibres, and another rootlet relatively few; so far as I have seen the variation is least in the thoracic region.

I shall have occasion to give measurements of the diameter of the myelinated fibres. The size of fibres depends to some extent upon the method of treatment. The sizes I give are those of the fibres of transverse sections of nerves slightly stretched and treated as mentioned

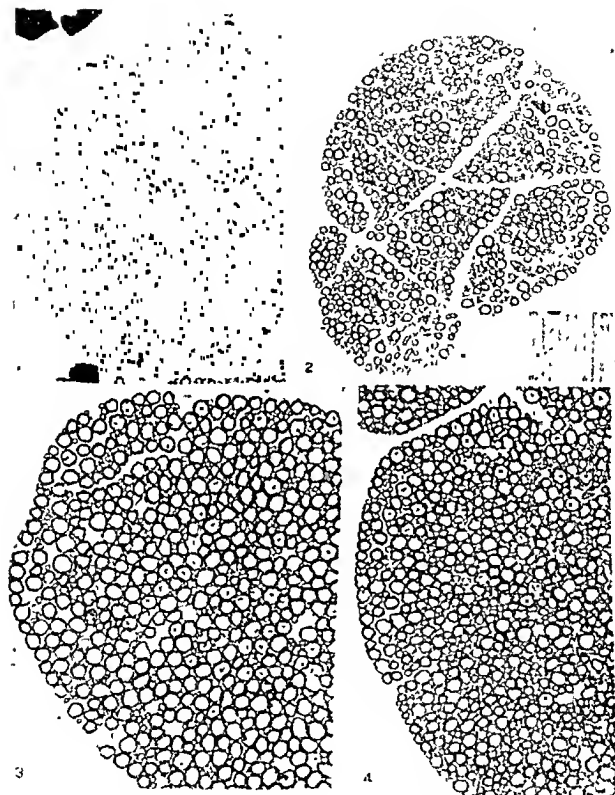


Fig. 1. Part of bundle of internal saphenous nerve. Many of the faint rings represent non myelinated fibres.

Fig. 2. Posterior rootlet of the 2nd sacral nerve showing the numerous fibres about 3μ in diameter (cp. with Fig. 4). The scale, 10μ , applies to all the figures.

Fig. 3. Part of anterior root of the 6th lumbar nerve having the maximum number of groups of 3.9 to 6 or 7μ fibres.

Fig. 4. Part of posterior root of the 6th lumbar nerve showing more equal number of fibres of different size than in the anterior root.

under 'Method'; the sizes were generally checked in teased specimens. In teased specimens a few of the smaller fibres were sometimes varicose; so that the measurement of a few of the smaller fibres in transverse sections could not be trusted. For these and other reasons I think slight differences in the size of the same class of fibre given by different observers are probably due to the mode of treatment. In general I find the great majority of the fibres of the white rami and of the cervical sympathetic in the cat to be about 3μ , but in some of my specimens made in earlier years, the majority were smaller, about 2.5μ . Some methods of treatment apparently greatly alter the size. Thus, Bidder and Volkmann⁽⁹⁾ found that the smallest fibres—which were mainly sympathetic—were $\cdot 00001$ Paris inch, i.e. 4.9μ .

Since nerve fibres of different size are not equally distributed either in a nerve trunk or in nerve roots, there is a large possible error when the average, maximal or minimal sizes of fibres in a nerve or nerve root are estimated by teasing out a portion of it. In consequence the data given by Bidder and Volkmann and by Schwalbe⁽¹⁰⁾ must be verified in transverse sections before it is safe to draw any deductions from them. The considerable variations given by Schwalbe of the minimal size of the fibres in the roots of the nerves of man do not occur in the cat, and it can, I think, be safely prophesied that they do not occur in man.

The breaking of the nerve fibres of the roots in teasing may be due to the neurolemma being more delicate than in peripheral nerves, or to the presence of Henle's sheath in the latter. In teased specimens of the roots, both anterior and posterior, it is common to obtain long stretches of axis cylinder projecting from the rest of the nerve; these show rows of small granules in an otherwise homogeneous substance and do not look as if they were formed of fibrillae. It may be noted also that the lipid constituent of the myelin is usually absent for 12 to 15μ on either side of a node, the distance varying somewhat in different nerve roots; in consequence the appearance of nodes is much more frequent in sections of the roots than in sections of the peripheral nerves.

The small nerve fibres of the posterior roots. Reissner⁽¹¹⁾ described all the posterior roots as having bundles of small nerve fibres between the larger ones and from that time to this, so far as I know, the constituents of all the posterior roots have been held to be practically the same. Reissner's 'small' fibres varied from 2 to 6μ . Apparently he fixed the nerves with chromic acid and stained them with carmine. Sections of the posterior roots of the nerves which do not give off efferent autonomic fibres when treated with osmic acid do not strike the eye as having

bundles of small nerves amongst large ones. The general impression is that of interspersed fibres of all sizes from 2 to 20 μ or more. On closer examination of the posterior roots it is seen that the fibres of 4 to 7 μ (about) are not infrequently in small groups; this is more distinct in the roots of the large nerves than in those of the small ones. It is seen also that the fibres of 2 to 3.5 μ (about) usually occur with others up to 7 μ . I have not counted the smaller fibres, but they are a much less conspicuous feature of the section than those of 4 to 7 μ , and in some of the rootlets they are barely represented.

The appearance changes in the upper thoracic region. The 2 to 3.5 μ fibres are much more numerous, as Gaskell showed was the case in the anterior roots. They occur, partly almost alone, partly mixed with 4 to 7 μ fibres. The proportion of 4 to 7 μ fibres to larger fibres appears to be much the same as in the roots of the cervical, lower lumbar and coccygeal nerves. In the 13th thoracic and the upper lumbar nerves, fibres of about 3 μ though in considerable number are much less conspicuous and there are many of 5 to 7 μ . In the 2nd and 3rd sacral, the 3 μ fibres are again conspicuous and some are present in the 1st sacral. Fig. 2 in the text shows the appearance of the posterior roots of the 2nd sacral; it will be seen that it differs in character from the posterior root of the 6th lumbar (Fig. 4 in the text) and from that of the posterior root of the 1st coccygeal nerve given by Dale (8).

The small myelinated fibres of the anterior roots. Reissner laid stress on bundles of small fibres (2 to 6 μ) as distinguishing the thoracic anterior roots from those of the cervical and lumbar regions, and Gaskell similarly laid stress on bundles of small fibres (1.8 to 3.6 μ) as distinguishing the anterior roots of the nerves which sent fibres to the viscera. I do not find that the grouping of small fibres in bundles is a distinguishing character. In some parts of the anterior roots of the lower cervical and lower lumbar nerves there are bundles of small fibres (up to 6 or 7 μ), and these are more obvious than in parts of the posterior roots (cp. Figs. 3 and 4 in the text). Further, in the nerves which send few fibres to the viscera, the small fibres (2 to 4 μ) are not more in bundles than other sizes of fibres. The grouping of fibres in bundles depends, I think, simply on their number.

It was probably the insistence on bundles that led Gaskell originally to consider that the 1st thoracic and 1st sacral nerves had no 'visceral' fibres. Until re reading Reissner's account for the purpose of this paper, I had not noticed that his 'small' fibres included those up to 6 μ , and this was not mentioned by Gaskell. Since Gaskell's bundles were of 2 to 3.6 μ fibres, he not only gave meaning to the difference in the anterior roots in the different regions, but was the first to describe the real difference.

The point I wished to satisfy myself about was whether fibres of the size of preganglionic sympathetic fibres occur in the anterior roots of those nerves in which I, and others, had found no preganglionic fibres by experimental and degeneration methods. Gaskell⁽⁷⁾ viewed the question from a broad point of view. He stated that there were bundles of fibres of 1.8 to 3.6μ in the nerves having white rami and that no fibre smaller than 3.6μ was present in nerves having no white ramus. This leaves an undetermined number of 3.6μ fibres common to the two sets of anterior roots. Moreover, the figure given by Gaskell of a nerve having no white ramus¹ (Pl. II, fig. 6) has a few fibres about 3μ in diameter, five of them forming a bundle. Reissner, previously, had described the anterior roots in the cervical and lower lumbar regions as having some fibres 2 to 4μ in diameter. Sherrington mentioned incidentally^(4, p. 234) that in the anterior root of the 7th lumbar nerve, a few fibres 2 to 3μ were scattered amongst the larger fibres. In the photographic reproduction of the anterior root of the 1st coccygeal nerve of the cat given by Dale⁽⁸⁾ there are a few fibres less than 3.6μ in diameter.

The surest criterion of the size of sympathetic fibres is afforded by examination of the cervical sympathetic since no afferent fibres have been found to run to this from the spinal cord. As I pointed out earlier⁽¹²⁾ the fibres of the cervical sympathetic occasionally contain no fibres larger than 4μ . I found the variation to be from 2 to 4μ . In the cases I have examined by the degeneration method, the few larger fibres, commonly present, came from the vagus. In my recent specimens; as in most of my earlier ones, the great majority of the fibres were about 3μ in diameter, 2μ and 4μ fibres were comparatively rare. In transverse sections of the cervical and lower lumbar anterior roots I find very few fibres of 2 to 3μ : the number varied from 3 to 12 . On teasing out a portion of the roots I sometimes found that a few of the small fibres were varicose, it is then possible that the 2 to 3μ fibres seen in transverse sections are the narrow parts of somewhat varicose fibres. On the other hand, there are in these roots a considerable number of fibres about 3.9μ in diameter. *i.e.* of the size of the larger sympathetic fibres. They give me the impression that they have a slightly thicker myelin sheath than the sympathetic fibres, but I am not certain of this. They occur in small groups made up of 3.9 to about 6μ fibres, and vary greatly in number

¹ This nerve was the 1st thoracic which commonly, at any rate, has a white ramus in the dog, but it is possible that with an unusually posterior arrangement of nerves, there is none.

in different rootlets. The part with the maximum number in the 6th lumbar root is shown in Fig. 3 in the text. In one part of the 7th lumbar root there appeared to be many smaller, but on measuring them under a high magnification they were found with very few exceptions to be more than 3μ .

Thus, the character which distinguishes the anterior root of a nerve which sends no fibres to the sympathetic is not that it contains no fibres of the size of sympathetic fibres, but that it contains none, or excessively few, of the smaller fibres (2 to 3μ) which occur in large numbers in the sympathetic.

I have examined some other nerves, but each in one case only, so that the following features which characterised them may require revision on further observation. The nerve to the inferior oblique has large fibres, but more medium and small than the muscle nerves of the limbs and trunk. The phrenic nerve has all sizes from about 16μ down, but relatively few of 4 to 5μ , and more non-myelinated fibres than other muscle nerves. The hypoglossal near its end, as the nerve to skeletal muscle, has few 3μ fibres, and this raises the question of the destination of the numerous 3μ fibres of its ganglion. The superior laryngeal has many 4 to 5μ fibres. The inferior dental branch of the 5th nerve has fibres of all sizes from about 16μ downwards, and 3.5 to 5μ fibres are frequent. It has non-myelinated fibres in small patches and a good deal of connective tissue, in the pulp nerve of the incisor tooth the fibres are chiefly 8 to 10μ and 3 to 4μ . The short ciliary nerves (as I have said I have only examined them in one animal) consist chiefly of 4 to 4.5μ fibres with a thin myelin sheath, thus differing from preganglionic sympathetic fibres, and of some fibres about 10μ .

REMARKS

1. From the facts given above it is clear that results obtained with regard to the existence of non-myelinated fibres by the silver impregnation method may lead to erroneous conclusions unless tested by other methods. The silver impregnation method no doubt brings out in those parts of the sciatic which consist chiefly of cutaneous nerve fibres numerous non-myelinated fibres as described by Ranson. But if it shows similarly numerous dark stained dots in the parts of the trunk which consist chiefly of motor fibres, or in the posterior nerve roots, the dots can only represent the axis cylinders of small myelinated fibres.

2. The results suggest that the non-myelinated axons of the small spinal ganglion cells described by Cajal and by Dogiel become myelinated outside the ganglion. We have seen that they do not enter the spinal cord as non-myelinated fibres, and peripherally of the sacral ganglion (which only I have examined) I do not find any large increase of non-myelinated fibres. It is not, I think, likely that they send a process to the grey rami since in earlier experiments (12) I found no reflexes on stimulating the central ends of the grey rami.

3. The degree of effect produced by nerve stimulation is in general at any rate proportional to the number of fibres stimulated. The scantiness of the non-myelinated fibres in the nerves to skeletal muscle indicates that control of the sympathetic on the blood vessels of muscle is less than that which it has on the vessels of the skin. In fact, stimulation of the sympathetic causes much less prompt pallor in skeletal muscle than in the skin. Since the non-myelinated fibres in the nerves to skeletal muscle supply the blood vessels, there can be very few for the muscle fibres themselves on the supposition that there are two distinct sets. If on the other hand it is held that the fibres divide, giving one branch to a blood vessel and another to a muscle fibre, they must undergo great division, and on the theory that they normally keep up tonic contraction in the muscle fibres, a stimulus causing contraction of blood vessels would be accompanied by an increase of tonic contraction. So far as these facts are concerned the simplest hypothesis is that the non-myelinated fibres do not supply the muscle fibres.

4. No explanation has been given of the extraordinary variation in size of the myelinated fibres. I would suggest that one of the main factors in determining size is the nature of the tissue in which the fibre ends. By this I do not mean that there is a single size for each, or for any, tissue, but that for each tissue there is a size which is most frequent and that the other fibres running to the tissue only vary within certain limits from the most frequent size. We have seen that large numbers of fibres about 3μ in diameter are present in the posterior roots of the spinal nerves sending fibres to the viscera, and similar fibres are known to be present in the vagus. These then may be regarded as afferent fibres for the unstriated muscle and glands of the viscera. Some, but relatively few, 3μ fibres are present in the posterior roots of the nerves which send fibres to the skin. By analogy these might be regarded as the afferent fibres of the unstriated muscle and glands of the skin, in which case there would be some ground for considering the 3μ class of fibre as the afferent fibre of the autonomic system. It may be mentioned that in the muscle nerves there are very few 2 to 3μ fibres and these run with non-myelinated fibres.

In the sympathetic, as I have previously pointed out⁽¹²⁾, a certain number of fibres of about 5μ and of about 8μ occur with a few of 10 to 12μ , and most at any rate of the larger fibres run to Pacinian bodies. Thus there is reason for supposing that the larger nerve fibres run to capsule end-organs whilst the 5μ class of fibre end in the connective tissue membranes of the viscera. In the pelvic nerve as shown by

Anderson and myself⁽¹³⁾ there are one or two dozen of the larger fibres, these may be taken as ending in the genital corpuscles, and several hundred of the 5μ class of fibres, which may run to the membranes and account for the greater sensitiveness of the pelvic than of the intestinal viscera.

The constitution of the depressor nerve is not necessarily out of harmony with this scheme. In an early paper⁽¹²⁾ I mentioned that the depressor nerve of the rabbit besides containing 3μ fibres contains about 30 of 4 to 7μ and a few of 8 to 10μ . Possibly the larger fibres are not constant, for Sarkar⁽¹¹⁾ in a recent communication gives the common size as 4 to 6μ and only in two nerves found two or three fibres of about 8μ . However this may be, the depressor supplies both unstriated and cardiac striated muscles; the 3μ fibres may be the afferent fibres of the former and the 4 to 7μ fibres those of the latter.

A decrease in the size of the fibres on their course from the spinal cord—apart from branching in the tissue—has been shown by several observers, especially by Schwalbe⁽¹⁰⁾ and by Sherrington⁽⁴⁾. The decrease only applies to the larger fibres, smaller fibres than those in the roots are not present in peripheral nerves. Thus it is difficult to be certain of the correspondence of fibres of a given size in the peripheral nerves with those in the roots. Sherrington has shown that most at any rate of the larger fibres in a muscle nerve end in the muscle spindles. I find that no fibres of the larger size of those in muscle nerves run to the skin. Thus the largest fibres whether of the anterior or of the posterior roots run to striated muscle. It is known that medium sized fibres run to the tactile corpuscles of the skin and it appears not unlikely that the fibres ending in capsulated organs in the skin are on the whole larger than those which end in the epithelium.

In the anterior roots the fibres which run to visceral striated muscle (the external anal sphincter and muscles of the larynx) are so far as I have seen of medium size (mostly 6 to 8μ); and it is known that medium sized fibres are in a great majority in the hypoglossal nerve. Fibres of 7.5 to 11μ are not numerous in the anterior roots of the spinal nerves. Thus whilst a characteristic of nerves of skeletal striated muscle is the presence of many large fibres, a characteristic of the nerves of visceral striated muscle is the presence of many medium sized fibres. In some rootlets of the anterior roots, small bundles of fibres about 4 to 7μ form a striking feature in contrast with the numerous large fibres (cp. Fig. 3 in the text), and it is, I think, probable that they end in a different constituent of the muscle. This constituent is not the "red" muscle fibre,

since the nerve to the soleus, though having somewhat smaller fibres than the other muscle nerves examined, has few of 4 to 5 μ . The small fibres may perhaps form the small nerve endings in muscle spindles, though it must be mentioned that Ruffini and others regard these as being formed by branches of the large nerves.

Whilst I think that the size of the fibres has some definite connexion with the kind of tissue in which the fibres end, it will require much detailed examination of different nerves to determine the exact connexion and the influence of other factors. There is also the possibility that some of the small afferent fibres lose their myelin on their course to the periphery.

CONCLUSIONS.

1. Cutaneous nerves contain many non-myelinated fibres, the nerves to skeletal muscle contain few. The result is not in favour of the theory that non-myelinated fibres have any considerable connexion with striated muscle fibres.

2. All anterior roots of the spinal nerves are distinguished from the posterior roots by their containing a relatively large proportion of fibres 13 μ and more in diameter, and a relatively small number of fibres of about 7.5 to 11 μ . These differences are most distinct in the lower cervical and lower lumbar regions.

3. The different rootlets of a nerve root vary in constitution, some have many small fibres—up to about 6 μ —and others very few. The arrangement of fibres in bundles depends chiefly on their number.

4. Very few non-myelinated fibres and probably none enter the spinal cord in the posterior roots.

5. In the posterior roots of the nerves, the anterior roots of which have no autonomic fibres, there are a considerable number of fibres about 5 μ in diameter, with a less number about 3 μ in diameter. In the posterior roots of the nerves, the anterior roots of which have autonomic fibres, there is a great increase in the number of the 3 μ fibres, but not in that of the 5 μ class of fibres. It is suggested that the 3 μ fibres in all the posterior roots are the afferent fibres of unstriated muscle and glands.

6. The anterior roots of the nerves which contain no autonomic fibres have fibres of the size of the larger preganglionic autonomic fibres (3.8 to 4 μ), but excessively few, if any, of the size of the smaller autonomic fibres (2 to 3 μ).

7. A large factor in determining the size of nerve fibres is the nature of the tissue with which they are connected.

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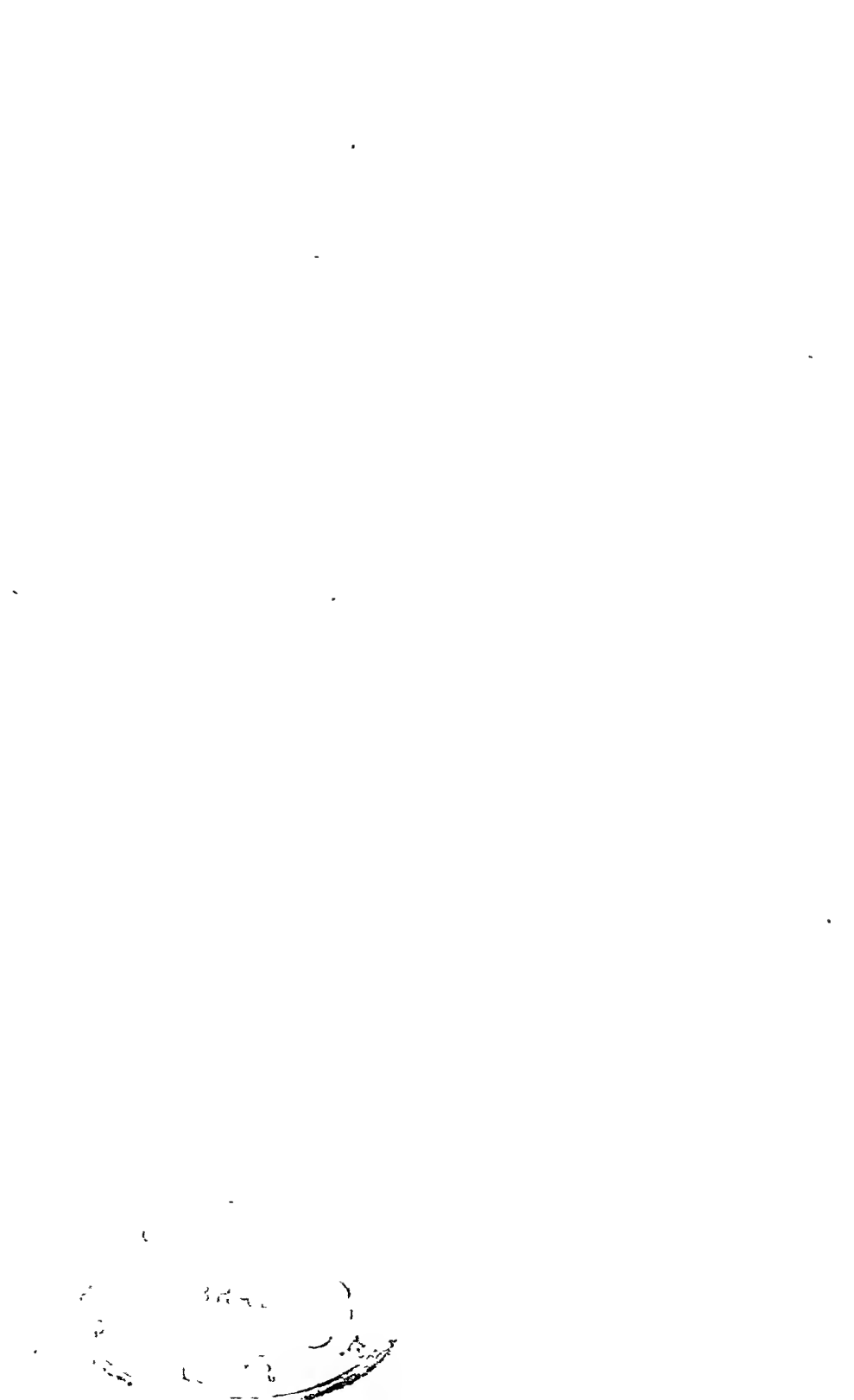
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DESCRIPTION OF PLATE I

The portion of the stage micrometer (10μ divisions) was photographed with the same objective, ocular and length of camera as the specimens in Figs 1-4 and in Fig 6

- Fig 1 A cutaneous branch of a primary dorsal division of a spinal nerve. The non myelinated fibres form compact masses with the endoneurium throughout the nerve
- Fig 2 A ventral cutaneous branch. This was taken a day after the death of the cat, and it was allowed to soak for five minutes in the (colourless) fluid which had accumulated in parts of the subcutaneous tissue. The axons of the non myelinated fibres in the specimen were stained slightly only, the sheath and connective tissue were stained brown, and small myelinated fibres black. In the microphotograph the myelin of the small fibres is only slightly darker than the sheath of the non myelinated fibres
- Fig 3 Part of nerve bundle supplying the mastic head of the gastrocnemius muscle showing the relative scantiness of medium sized myelinated fibres and the greater scantiness of 2 to 3μ fibres and of non myelinated fibres. The finer strands of the endoneurium are not reproduced.
- Figs 4 and 6 Portions of the 6th lumbar posterior root
- Fig 5 Portions of the 7th cervical posterior root. All that was visible under the microscope is reproduced, except a few very fine filaments of connective tissue. Magnification as in the text figures





THE ORIGIN OF THE ELECTRICAL CHANGE IN
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IN 1913 Mines⁽¹⁾ suggested that the liberation of lactic acid is largely the cause of the action current of muscle. In 1914 Hill⁽²⁾ showed that increasing the frequency of excitation, up to the limit when complete tetanus results, never decreases but almost invariably, sometimes largely, increases the heat-production of muscle. After complete tetanus is reached the heat-production per second is independent of the frequency of excitation. In 1921, Hartree and Hill⁽³⁾ determined the relation between the heat-production in a tetanus and the duration of the stimulus. They found that in a prolonged contraction there is an extensive outburst of heat as the result of the first moment of the stimulus, and that successive equal periods of stimulation produce progressively smaller effects until the heat produced per second of stimulation attains a constant value. By employing ballistically a high resistance sensitive galvanometer of long period, it is possible to record, for any time of stimulation up to (say) five seconds, the total amount of electricity sent round a circuit connected to an injured and an uninjured spot on the muscle, the injury current being balanced. There is every reason to suppose that the heat-production runs parallel⁽⁴⁾ to the liberation of lactic acid. If therefore the supposition be correct that the electrical change in muscle is due to the liberation of lactic acid, then the total electrical change measured as above ought to behave in the same way as the heat-production, if the experiments are performed in the same manner. To test this the following experiments were carried out.

Method. A sensitive, moving-coil, mirror galvanometer, type L 115 (Cambridge and Paul Instrument Co., Ltd) was used. This instrument had a long period and a resistance of approximately 2099 ohms. To avoid stray currents the galvanometer was mounted on vulcanite blocks, and all the instruments (with the observer) were insulated by similar means.

The muscle chamber was that used by Hill in his earlier experiments on the heat-production of muscle⁽⁵⁾. It was fitted with a vulcanite block and a screw clamp. The femoral end of the gastrocnemius muscle was

In the experiments in which the duration of the stimulus was varied readings were taken with increasing periods, and repeated with decreasing periods of stimulation, and an attempt made to eliminate the effects of fatigue by taking the mean of the two readings of any one period.

In experiments in which the frequency of the stimulus was varied, the arms of the contact breaker were set to give a stimulus of approximately one second. A connection was made with one segment of the distributor of the magneto, so that only one shock in eight was given to the nerve. The nerve was stimulated and the reading of the galvanometer taken. Readings were then taken using two, four and eight segments of the distributor. The speed of rotation of the magneto was measured by a revolution counter and stop watch.

The magneto was driven at different speeds by changing the belt on the pulleys, readings at each speed being taken from the galvanometer. In every experiment, readings were taken first with increasing and then with decreasing frequencies, and the mean taken of the readings at any one frequency, to eliminate the effects of fatigue.

The experiments described above were repeated at various temperatures. After a change of temperature, the muscle was allowed to remain at rest for 30 minutes so that its temperature might approximate to that of the chamber. In all experiments the results are given in arbitrary units for galvanometer deflection.

The relation between the duration of the stimulus and the total electrical change.

The experiments of which Fig. 3 is typical were repeated at various temperatures as shown in the upper curve of Fig. 3. In all cases the total electrical change was proportional to the duration of the stimulus. It was found that the muscle, which had necessarily to be injured at one end, died too rapidly to allow experiments to be made on one muscle at more than one temperature. Different muscles therefore were used in these experiments, so that nothing can be gained from a comparison of the slopes of the line at different temperatures.

Hartree and Hill⁽³⁾ investigated the relation between the total initial heat production and the duration of the stimulus under similar conditions, and a comparison of the curves they obtained with those in Fig. 3, will show that the two sets of curves bear little resemblance to one another. The heat-production is not a linear function of the duration of the stimulus though it becomes linear if the stimulus is continued; the linear portion however, if produced backwards, does not pass through

the origin. As there is every reason for assuming that the heat-production is proportional to the production of lactic acid, it would appear

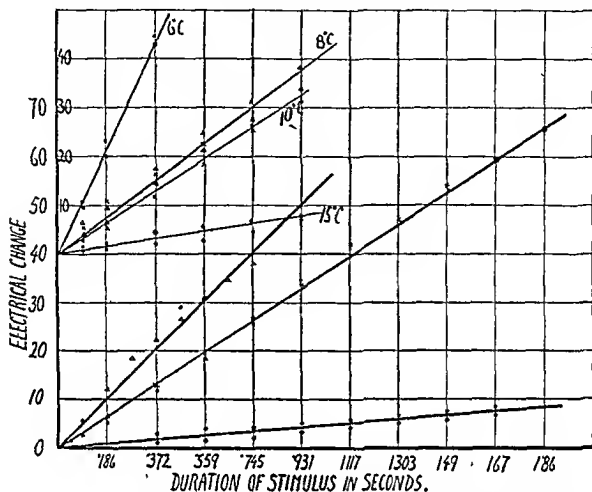


Fig. 3. Temp. 15° C. Lower curve. Experiments in October and November. Upper curve with ordinates 0 to 50. Experiments in January and February.

therefore, in contradiction to the hypothesis suggested by Mines(1), that the electrical changes which occur in a muscle on contraction do not bear any quantitative relation to the production of lactic acid.

The relation between the frequency of stimulation and the total electrical change.

Within the limits dealt with in these experiments, the total electrical change in a stimulus of fixed duration is proportional to the frequency of stimulation.

Typical results are shown in Fig. 4. Hill(2) found, under similar conditions, that increased frequency of stimulation caused increased heat-production up to the limit when complete tetanus resulted—beyond this point however, which occurs well within the range of the linear relation of the total electrical change to frequency, the heat-production was independent of the frequency of stimulus. Hence the heat-production

and the total electrical change do not bear the same relation to the frequency of stimulation, therefore the total electrical change is not

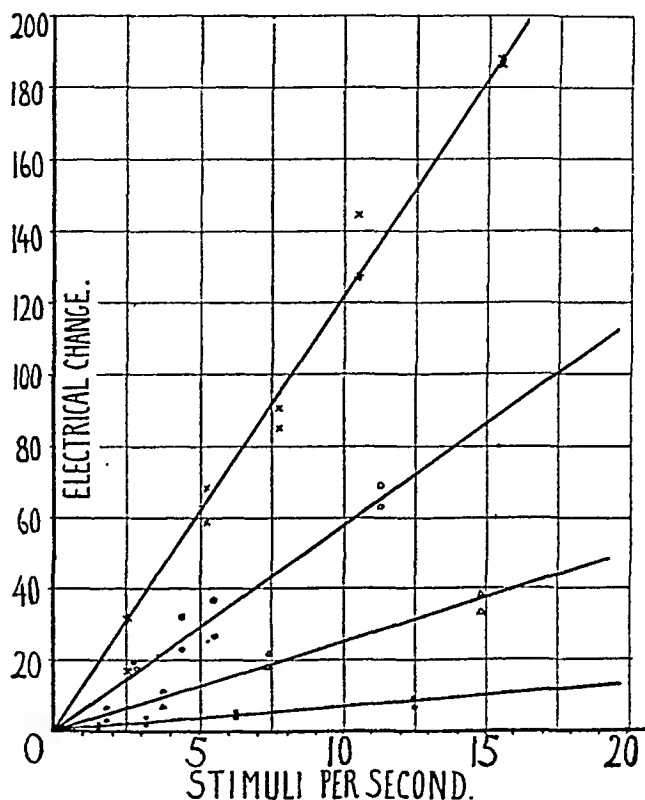


Fig. 4. Temp. 15° C. Period of stimulus 1 second. November and January.

quantitatively proportional to the production of lactic acid. The linearity of the relation between the total electrical change and the frequency of stimulation is not affected by temperature, and gave similar results to Fig. 3.

CONCLUSIONS.

By employing a sensitive galvanometer of long period, connected to an injured and an uninjured spot on a muscle suspended isometrically and stimulated through its nerve, it is possible to sum the electrical effects accompanying a prolonged contraction. If as Mines suggested the electrical change in muscle be caused by the production of lactic acid, the summed electrical effect should bear to (a) the duration and (b) the frequency of the stimulus, the same relation as does the production of lactic acid. On many grounds the production of lactic acid in

muscle may be expected to run parallel to that of heat: we show experimentally that the relations between the total electrical change and (a) the duration, (b) the frequency of the stimulus are different from those established by other authors between the heat-production and the same two variables, and we conclude that the production of lactic acid is not the prime cause of the electrical change.

Our best thanks are due to Professor A. V. Hill for his help and suggestions.

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REPRODUCTION IN CELL-COMMUNITIES¹.

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The allelocatalytic effect. It was pointed out by McKendrick and Pai⁽¹⁾ that the rate of multiplication of bacteria is for a time proportional to the concentration of the food material and to the number of the organisms; and that later the multiplication becomes slower and finally stops. The inference seemed to be that the multiplication is governed by the amount of food available. I have shown⁽²⁾ that pairs of infusoria (*Enchelys farcinem*), after a "lag" period, mutually accelerated one another's reproduction in media which contain or have contained bacteria. Thus each formed some product which increased the rate of reproduction of the other provided a bacterial product which I called the X substance was present. The X substance increased the reproduction rate of single infusoria and I concluded that the accelerating substance was produced from it, or through its agency, by the infusoria. Peters⁽³⁾ has recently found that infusoria (*Colpidium*) can multiply indefinitely in media containing soluble food material in the absence of bacteria and their products. Thus the accelerating effect of bacterial products might be due either to their supplying food or to their containing an accelerating agent. It is I think due to both and there is evidence that bacterised infusions contain an accelerating agent^(4,5). The two modes of action cannot unfortunately be distinguished in this case, but an approach towards their separation has been made in the case of yeast. If compressed yeast be immersed in ether to cytolyse the cells, and then dried on a water-bath and ground up to a fine powder in a mortar, the addition of a small proportion of this powder to hay infusion greatly accelerates the reproductive rate of infusoria isolated into it. Autolysed yeast extract has a like effect. But if the yeast be thoroughly and repeatedly extracted with cold acetone, the residue is devoid of accelerative effect. We can hardly suppose that all soluble materials of a nutritive character have been removed from the yeast by this procedure, but the substance

¹ The expenses of this investigation were defrayed by the Animal Products Research Foundation of the University of Adelaide.

which is responsible for the accelerative effect obviously has been removed.

I have made some further experiments upon the accelerative effect which infusoria have on one another; it may be spoken of as the "allelo-catalytic" effect. It is clearly shown by the increase in reproduction rate which is brought about by mere reduction in the volume of the culture medium.

TABLE I.

Single infusoria were isolated into fresh "normal hay infusion" from two six-day-old parent cultures, A and B, which were thickly inhabited. After washing by re-isolation, they were separately placed in varying volumes of the hay infusion. After 44 hours the counts in the various cultures were as follow:

Culture	Weight of culture fluid mgms.	Number of individuals after 44 hours	Number x weight
A. 372	5.0	250	1250
374	10.0	31	310
375	13.6	104	1414
373	23.4	64	1498
376	34.6	33	1142
B. 372	1.4	64	90
373	7.6	4	30
375	13.0	8	104
374	23.8	6	143
376	32.2	4	129

Thus, if we except the irregular results obtained from cultures 374 A and 373 B, it is evident that the population attained in these cultures in 44 hours was very nearly inversely proportional to their volume, and if in the A series a volume of about 1.5 c.c., or in the B series about 0.15 c.c., had been employed, we may infer that no multiplication at all would have occurred. As a matter of fact, single individuals isolated into volumes exceeding 1 c.c. very rarely survive, and failures to reproduce on the part of individuals isolated into cultures exceeding 0.1 c.c. in volume are not at all infrequent. This phenomenon has also been observed by Peters in the cultivation of *Colpidium* (3) and by Pasteur and others in the cultivation of yeasts. We must infer that some necessary substance is dissipated by the infusoria into the surrounding medium and that reproductive rate is nearly proportional to its concentration. When this substance is too dilute, i.e. is dissipated into too large a volume of medium, reproduction, and even maintenance, become impossible.

When an infusorian has been isolated into fresh "normal hay infusion" or into distilled water to which a suitable reaction and tonicity have been communicated by alkaline salts, the presence of an auto-catalyst of cell-multiplication in the medium can be demonstrated,

directly after the first cell-division, by isolating one of the daughter-cells into a fresh medium. The reproductive rate of this re-isolated cell is always much less than that of the cell which has been left undisturbed in its surrounding medium, and this is true even when the isolated cell has been transferred from a medium poor in foodstuffs, such as faintly alkalisied distilled water, to one which is comparatively rich in foodstuffs, such as normal hay infusion.

Finally, glass-distilled water which has been rendered faintly alkaline by the addition of 1 c.c. of $N/10$ Na_2CO_3 solution per 100 c.c.¹, and which has been inhabited by multiplying infusoria for 48 hours, may be shown to contain an agent which accelerates the reproduction of infusoria in the following manner: The water is freed from infusoria by heating to 50°C. to immobilise them, followed by filtration through a double filter paper. The filtrate is then neutralised to cochineal indicator by the addition of $n/100$ hydrochloric acid and evaporated on a boiling water-bath to one-half its original volume. A small precipitate of coagulated protein is removed by filtration and the fluid, stored in flasks plugged with cotton, is boiled several times at intervals of two or three days to sterilise it. This fluid, when added to "buffered" distilled water or to normal hay infusion, greatly accelerates the reproductive rate of infusoria isolated into these media.

For reasons which will be clear in the sequel, the phenomenon of "lag," taken together with the autocatalytic character of their multiplication, indicates that bacteria, as well as infusoria, discharge into the surrounding medium a material capable of accelerating their own reproductive rate, while the "bios" phenomenon of Wildiers(6,7) may indicate that similar substances are produced by all kinds of living matter. It is probable that we are here dealing with some universal process which underlies and determines the multiplication of all types of cells and to which the autocatalytic character of growth in widely divergent types of living matter is attributable.

The autocatalytic character of reproduction in cultures of infusoria has thus been traced, in part to the inherent capacity of each cell to reproduce itself, due to the presence within it of a catalyser capable of effecting protoplasmic synthesis, and, in part to the shedding of a catalyser into the surrounding medium, through the agency of which the cells are enabled mutually to facilitate each other's reproduction.

Turning, now, to the question of the nature of the retardative influence which sets a limit to the population of any cell-community. If the

¹ This however exceeds the optimum alkalinity.

infusoria are removed from an old hay infusion culture, by heating to a temperature somewhat above their thermal death-point (50°C) and then filtering through two or three thicknesses of paper, it is found that this filtered, infusoria-free culture medium, which has been inhabited by a maximal population of infusoria for a prolonged period (for example, one month), is nevertheless capable of supporting the multiplication of infusoria isolated into it. Indeed, if these infusoria are derived from a relatively young culture, little or no diminution of reproductive rate in comparison with that in fresh hay infusion is observed. It is evident that for such infusoria the medium contains no substances which inhibit multiplication.

If, instead of inoculating this old culture medium with an individual derived from a young and vigorous culture we employ one of the individuals which originally inhabited the old culture fluid, very different results are obtained. To secure a fair comparison of reproductive rates we must not isolate such individuals into fresh undiluted "normal hay infusion" because, during long inhabitation by infusoria and bacteria, old culture media become hypotonic in comparison with fresh hay infusion, so that individuals isolated from very old cultures into "normal hay infusion" lose water and become laterally compressed and disc shaped. Such a transfer is very badly endured and the individuals thus treated sometimes die, or, more usually, undergo an exceptionally prolonged "lag period." On the other hand transfer to a relatively hypotonic medium is generally well endured. It was found that "normal hay infusion" diluted with four times its volume of distilled water containing two volumes per hundred of $m/15$ phosphate mixture ($\text{pH} = 7.7$) provided a medium which was neither hypertonic nor markedly hypotonic to infusoria isolated from culture fluids which were thirty or forty days old.

The rate of reproduction of individuals isolated from these old cultures is always far inferior, when they are isolated into the old culture medium itself, to the reproductive rate displayed by similar individuals isolated into hay infusion of similar tonicity, and usually even to the reproductive rate which is displayed in undiluted hay infusion, in spite of the handicap of hypertonicity. The maximal population is also soon attained in the old culture fluid and is very scanty in comparison with the population which is attained in the fresh infusion, with or without dilution. It is also inferior to the maximal population attained in the old culture fluid when it is inoculated with an individual derived from a young culture. This fact, and the fact that maximal, reproductive rate of individuals

from young cultures is possible in the same medium, forbids the supposition that it contains any toxic substance. Despite the fact that dilution must result in diminution of the available nutrients, the maximal population attained in such cultures may actually somewhat exceed that attainable in undiluted cultures. The following experiments are illustrative, one individual from a forty-day old culture being in each instance isolated into the same volume (about 0.15 c.c.) of the culture media named.

TABLE II.

Culture	Culture fluid	Individuals after			
		24 hours	48 hours	72 hours	96 hours
490 A	Old culture medium	3	31	—	250
490 B	" " " " " " " " " "	4	47	—	207
491 A	Old culture medium + equal volume of buffered distilled water	5	171	—	234
491 B	" " " " " " " " " "	4 $\frac{1}{2}$	356	—	350
493 A	Distilled water pH=7.7	1	—	4	35
493 B	" " " " " " " " " "	1	—	34	118
494 A	Buffered "distilled" water 5 vols., normal hay infusion 1 vol.	2	—	2000	—
494 B	" " " " " " " " " "	2	—	4000	—

In the light of the results given above, we cannot attribute the failure of individuals inhabiting an old culture to reproduce indefinitely, either to loss of reproductive capacity of the cells themselves or to inability of the culture fluid to sustain any larger population. The cessation of reproduction is evidently due to a reciprocal relation between the old culture fluid and the individuals inhabiting it, whereby the fluid is no longer suitable for the reproduction of these particular individuals.

The growth of individual cells. The question arises whether the cell retains any of the accelerating agent. If it does we should expect the development of each individual cell to display the time-relations of an autocatalysed reaction. It was pointed out by Loeb(8) that the production of nuclei in the early stage of development is essentially an autocatalytic process. The investigations of Popoff(9) have revealed very characteristic autocatalytic time-relations in the development of the individual nucleus. According to this author, nuclear growth during the period immediately succeeding cell-division is considerably slower, relatively, than cytoplasmic growth, so that for a period the nuclear ratio diminishes. While cytoplasmic growth continues at a uniform rate, however, the rate of growth of the nucleus suffers progressive acceleration and finally becomes much more rapid, relatively, than the growth of the enveloping cytoplasm. Thus the nuclear ratio rises until it attains a characteristic critical ratio at which cell-division occurs and

the cycle is reinaugurated. These results of Popoff have been substantially confirmed by Hertwig⁽¹⁰⁾ and by Conklin⁽¹¹⁾. The facts lead us to infer that the nucleus contains a certain portion of the accelerative agent. With certain special exceptions, the growth of cytoplasm is obviously determined by the multiplication of the nuclei. Hence, if the production of nuclear materials in a multicellular organism, or in a culture of unicellular organisms, is autocatalysed, the total production of protoplasm must display autocatalytic time-relations.

The significance of the lag period. It is a fact which has been noted by all observers, that an isolated cell placed in a nutrient medium displays a period of inertia or "lag" before it responds to the stimulus of abundant foodstuffs by reproduction of its kind. We may therefore safely infer that this is a universal property of living matter, and an inevitable consequence of isolation.

Now I have repeatedly observed that the effect of accelerative agents upon the multiplication of infusoria is not manifested to any important degree during the period of "lag." Thus two individuals isolated together into the same drop of hay infusion do not begin to divide any earlier than a single individual isolated into a drop of similar size. But once division has occurred acceleration begins to manifest itself. Similarly the lag periods of single infusoria isolated into varying volumes of culture fluid do not appreciably differ, but once cell division has occurred then the intervals between successive divisions are much abbreviated in the smaller volumes of culture fluid and multiplication is correspondingly accelerated. The accelerative agent which is discharged by multiplying infusoria into "buffered" distilled water does not abbreviate the lag period of infusoria isolated into nutrient media which contain it. Again, yeast extract shortens the lag period only to a relatively insignificant degree while, subsequently to the first division, the reproductive rate is decidedly enhanced. I have observed the same phenomenon in connection with the acceleration of the multiplication of infusoria which is induced by cholesterol and by tethelin.

We must infer from these observations that the isolated cell is relatively insusceptible to agents accelerating nuclear synthesis during the period preceding the first division. For the time being the nucleus is closed to outside influences and carries with it the characteristics of the nuclei of the cells which inhabited the parent culture. If the parent culture is old and densely populated, reproduction has ceased in it, and correspondingly we find that the lag period is immensely prolonged. If, on the contrary, the parent culture is a youthful one in which a high

reproductive rate is still maintained, then the lag period is brief. If the lag period be deducted from each, the reproductive rate of individuals isolated from an old parent culture is not less, but may actually be greater than the reproductive rate of individuals isolated from a young parent culture. Hence cultures which are initially very slow to develop, generally display the most striking autoacceleration. Similarly the lag period in bacterial cultures is much more prolonged when the parent culture is old⁽¹²⁾, and *in vitro* cultures of vertebrate tissue display a much longer lag period when the isolated cells are derived from adult tissues than when they are derived from embryonic tissues^(13, 14). The principle that the lag period increases with the age of the parent cell-community appears, therefore, to be universally applicable.

These considerations, taken together, unite in indicating pretty clearly that the time of distribution of accelerative agents between the nucleus and the surrounding medium is that at which cell division or the preceding nuclear division occurs. And this is, indeed, a very reasonable assumption, for the nuclear membrane is known to be relatively impermeable to many soluble materials⁽¹³⁾, and its dissolution removes this barrier.

The fact that the synthesis of individual nuclei is autocatalysed shows, indeed, that the accelerative agent cannot issue from the nucleus during the period preceding nuclear division. It is also a highly suggestive fact that the rate of nuclear growth, which attains a maximum immediately before nuclear division, falls to a minimum immediately thereafter⁽¹⁴⁾. It does not appear possible to interpret this fact except by supposing that during the process of division the nucleus has lost some constituent which was responsible for the high rate of nuclear growth just before division occurred.

We are thus led to the formulation of the following hypothesis: During the periods between nuclear divisions, each nucleus retains the charge of autocatalyst with which it was originally provided and adds to it in the course of the nuclear synthesis which is rendered possible by its presence. At the next division the autocatalyst is shared between the nuclear materials and the surrounding medium in a proportion determined in part by its relative solubility in the two media, and in part by its affinity for chemical substances within the nucleus. At the end of this redistribution the autocatalyst stands in equilibrium between the external solvent medium, on the one hand, and on the other hand with the nuclear substances with which it combines or in which it is dissolved. The nuclear membrane is then re-formed, and the autocatalyst within

the nucleus is again shut off from dispersal into the surrounding medium until the occurrence of the next succeeding division.

This hypothesis, besides accounting for the facts described in this paper, throws a clear light upon a number of otherwise obscure and apparently unconnected phenomena. Thus the fact originally observed by Pasteur, and recently emphasised by Wildiers(6), and again by Peters(3), that a single cell cannot survive if isolated into too large a volume of fresh culture medium, is obviously traceable to the excessive loss or dilution of the nuclear autocatalyst which occurs directly the nuclear membrane is broken down. The acceleration of cellular multiplication by almost any living tissue extract—the “bios” effect of Wildiers—is obviously due to the prevention of this drainage from the nucleus by previous saturation, or partial saturation, of the surrounding medium with autocatalyst. The fact that in a suitable volume of culture medium relatively slow growth is possible in the absence of added “bios” or autocatalyst, is attributable to the fact that the autocatalyst is produced by the cells themselves and the medium ultimately becomes charged with it in consequence of their successive divisions.

SUMMARY.

1. As I have shown previously, when two infusoria (*Enchelys farcinem* Ehr.) are isolated into the same drop of culture medium, the rate of production of new cells is much more rapid than it is when a single individual is isolated into a drop of the same size. It may be sixteen times as great. This has been designated “*allelocatalytic effect*.”

2. The smaller the volume of culture medium into which a single infusorian is isolated, the greater is the initial multiplicative rate of the culture.

3. Distilled water, to which a proper reaction and tonicity have been communicated by the addition of a “buffer” mixture of phosphates, and which has been densely populated by dividing infusoria, contains a substance which enhances the multiplicative rate of isolated infusoria.

4. The autocatalytic character of reproduction in cultures of infusoria is thus attributed, in part to the inherent capacity of each cell to reproduce itself, due to the presence within it of a catalyser capable of effecting protoplasmic synthesis, and in part to the shedding of a catalyser into the surrounding medium, through the agency of which the cells are enabled mutually to facilitate each other's reproduction.

5. An old culture-fluid contains no substances which are toxic for infusoria, nor does it retard the multiplication of infusoria isolated into

it from young cultures. Nevertheless, infusoria from old cultures develop much more slowly in old culture fluid than in fresh isotonic hay infusion, and the maximal resultant population is very scanty. Mere dilution of the old culture fluid with an equal volume of properly buffered distilled water enhances the reproductive rate of the individuals restored to it, and does not reduce the maximal attainable population.

6. During the "lag" period which succeeds isolation of an infusorian into a fresh culture medium, and before the first division has occurred, the organism is insusceptible to the action of agents which accelerate multiplication. The acceleration of reproductive rate is observable only after division of the isolated cell.

7. The accelerative agent in cellular multiplication originates in the nucleus, and the autocatalytic time-relations which distinguish all types of growth are referable to the fact that nuclear synthesis is autocatalysed.

8. It is suggested that during the periods between nuclear divisions each nucleus retains the charge of autocatalyst with which it was originally provided, and adds to it in the course of the nuclear synthesis which is rendered possible by its presence. At the next division the autocatalyst is shared between the nuclear materials and the surrounding medium in a proportion determined in part by its relative solubility in the two media, and in part by its affinity for chemical substances within the nucleus. At the end of this redistribution the autocatalyst stands in equilibrium between the external solvent medium, on the one hand, and on the other hand the nuclear substances with which it is combined or in which it dissolves. The nuclear membrane is then re-formed, and the autocatalyst within the nucleus is again shut off from dispersal.

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NUTRITION ON HIGH PROTEIN DIETARIES. BY
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It is generally agreed that protein can serve as the sole source of energy for the animal and that it can form carbohydrate and fat in the organism. Lusk⁽¹⁾ quotes Pflüger's experiment in which a dog was kept in active condition on meat alone. It is therefore reasonable to think that a diet consisting largely of protein supplemented with inorganic salts and those dietary factors (vitamins), the importance of which was unknown to the earlier workers, would suffice for the nutrition of the normal organism during the whole of its life cycle. It is of course apparent that such a diet is adequate for many of the carnivora.

Nevertheless, opinions have been repeatedly expressed that a high protein dietary is deleterious to omnivora or herbivora. We need scarcely deal in detail with the well-known views of Chittenden and his school but would refer briefly to the less known work of Chalmers Watson and his collaborators⁽²⁾. From a prolonged experimental investigation mainly on rats Watson concluded that a high protein diet is harmful because the growth of the young animal is impaired and in the mature animal certain organs and tissues develop abnormally or become damaged, e.g., hypertrophy of the thyroid, degeneration of the renal tubules. In reading through his papers, however, we were impressed by the similarity of the abnormalities he observed to those characteristic of certain vitamin deficiencies. We therefore decided to determine the effect of diets rich in proteins but containing in addition adequate amounts of salts and of the known accessory food factors.

Our experiments were carried out mainly on young growing rats as omnivorous animals and later on kittens as carnivorous animals. The rats and kittens were selected from normal litters so that some animals of each litter were on the high protein diet while the others were placed on normal diet to act as controls.

In the case of the rats the diets employed were as follows:

	Normal diet	High protein diet
Caseinogen	20 parts	83 parts
Starch	50	—
Yeast extract	5	5
Lemon juice	5	5
Butter ¹	15	—
Shark liver oil	—	2
Salt mixture	5	5
	100	100

Water *ad lib.*

In the high protein diet the quantities of yeast extract supplying the vitamin B, shark liver oil supplying the vitamin A, and lemon juice supplying the vitamin C had been proved by former experiments to be adequate for growing rats fed on a mixed artificial diet otherwise devoid of vitamins. The rats were given these dietaries when they had attained about 40 gms. weight (*i.e.* 20–30 days old) and in both cases consumed the diets readily, the average consumption being about 15 gm. dry weight per day. Fig. 1 shows the growth curves of the two groups of rats from the same litters when fed on these diets.

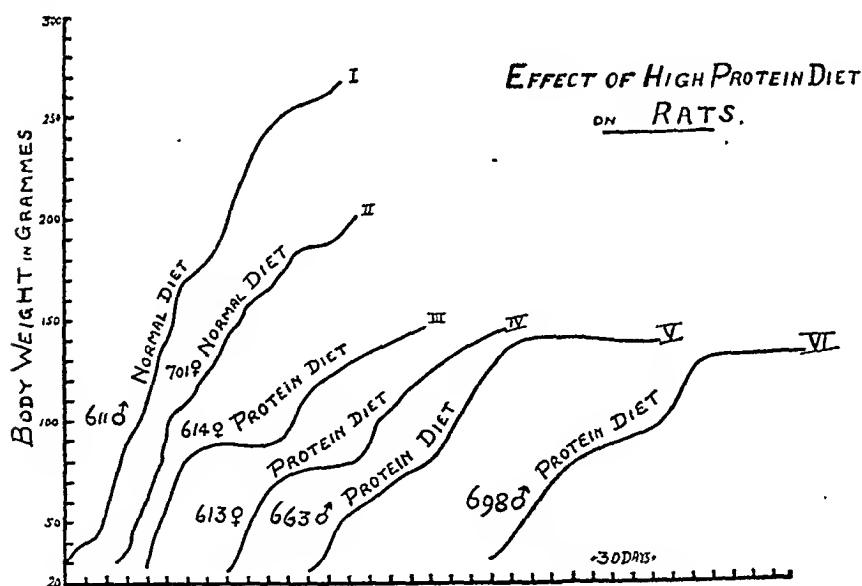


Fig. 1.

The rats on the balanced diet showed the normal growth that was expected, and at first the parallel group on the high protein dietary

¹ A later series of controls on a similar dietary in which the 15 p.c. of butter was replaced by 2 p.c. shark liver oil showed equally normal growth.

showed the same behaviour. They nearly trebled their weight before any abnormality was detected, which tends to confirm the preliminary results recorded by Osborne and Mendel(3). After about 30 days, however, they showed a retardation of growth. There is usually a slight temporary retardation of growth about this point in the normal growth curve, which probably represents the intermediate stage between two of the growth cycles studied by T. B. Robertson(1), but it appears to be much intensified in the case of our rats on the high protein dietary. After this period the latter group resumed growth but at a subnormal rate, although to outward appearances their condition and health were excellent.

The experiments were continued sufficiently long to allow for reproduction to take place and the group on the balanced diet produced and reared normal litters. Those on the high protein diet did not, however, show any disposition to breed. After four months feeding rats representative of both groups were killed for examination. The following table gives the weights of the control and experimental rats when 30 days old and when 130 days old, *i.e.* after feeding on normal and high protein diets for 100 days.

TABLE I

Exp.		Weight at 30 days grms	Weight at 130 days	
			Normal diet grms.	High protein diet grms.
A. 1	♂+♂+♂+♂	42	257	—
2		42	221	—
3		36	—	139
4		38	—	144
B. 1	♂+♂+♂+♂	41	250	—
2		42	182	—
3		45	—	139
4		42	—	121
C. 1	♂+♂+♂	36	—	146
2		35	—	116
3		40	148	—
D. 1	♂+♂	42	197	—
2		41	—	138

The letters A, B, C, D signify that the animals under each letter were of the same litter

From the above table the average weights at the age of 130 days are as follows:

Male rats normally fed, 242 grams. High protein diet, 142 grams.
 Female rats " 175 " " " " 135 "

Although the groups are rather too small to use for deducing averages it is interesting to note that the male rats on high protein diet had only attained 58.6 p.e. of the weight of the normally fed male rats, while the female rats on high protein diet reached 75 p.e. of the weight of the

control female animals. Table II shows the relative increase in body weight of the male and female rats of both groups:

		TABLE II. Relative body weights			
		Start	1 month	2 months	3 months
Normal.	Male	1	2	4	5.6
	Female	1	2.5	3.5	4.0
High protein.	Male	1	1.9	2.4	3.2
	Female	1	1.8	2.3	3.1

These figures show that in both groups male and female rats grew almost normally until the end of the first month, but later show the retardation which Chalmers Watson observed.

At the conclusion of the experiments the animals fed on high protein diet looked well, their coats were in good condition and their appetites were excellent; in fact they resembled normal animals younger than the controls. Detailed post mortem examinations were made of a number of the rats. In the high protein group plenty of body fat was found but the ribs showed marked beading as compared with the normal. The organs from rats of both groups were removed, weighed and their weights compared with the figures given by Donaldson(5) for the normal weights of organs for a given body-weight. The weights of the supra-renals, thyroid, ovaries, testes, spleen, liver and kidney in both sets were within normal limits of variation and microscopical examination showed no marked uniform difference between the experimental and control animals. The result confirms our suspicion that the pathological conditions in Chalmers Watson's rats were due to causes other than the high intake of protein. With regard to the beading of the ribs we hesitate to ascribe it to the high protein dietary, nor do we care to commit ourselves to an opinion as to the cause. It is well known that certain types of dietary deficiency tend to produce abnormal conditions at the costochondral junctions.

It was noticed that in some cases the contents of the abdominal cavity in the protein-fed rats had a markedly objectionable odour although they were freshly killed. It is possible that this was due to the exaggerated action of protein-putrefying organisms flourishing on the unusually large amount of protein products in the contents of the intestine.

In view of the normal condition of practically all the tissues of the high protein group it was difficult to explain their failure to grow. We first studied whether their daily consumption of the diet was sufficient to allow both for tissue building and for energy. Calculations showed that the average daily ration supplied approximately 60 cals. per 100 gm.

rat whilst the controls were consuming about 65 eals. per day. Previous experiments in this laboratory have taught us that rats of this weight can be maintained on 25 eals. per day and show normal development on 45 eals. It was therefore unlikely that the high protein group were showing retarded growth from an insufficient caloric intake.

The normal growth and condition of the rats fed on the mixed diet showed that the intake of the so-called vitamins was ample, and we therefore assumed that the same proportions of the ingredients of the food mixture which supplied these substances would be adequate for the high protein diet. To determine whether the failure to grow was a consequence of an omnivorous species being given a diet unnaturally high in protein we decided to study the question on carnivora which naturally subsist on such a food supply. The number of these experiments was small but the results were in general agreement.

Four kittens of the same litter were selected at the age of about six weeks. A male and female were used as controls and two females were fed on a high protein ration. The daily rations were made up as given below:

Normal diet	High protein diet
Raw beef (fat and lean) 110-170 gm.	Raw lean beef 110-170 gm.
Bread and milk <i>ad lib.</i>	Yeast extract 2-4 gm.
	Shark liver oil $\frac{1}{4}$ c.c.
	Salt mixture 2 gm. ¹

The high protein ration was compounded by trimming the fatty tissue from lean beef, mincing and mixing the addenda to supply the salts and vitamins. Both lots of cats consumed their diets well and those on the normal ration grew and showed absolutely normal behaviour. The cats fed on the high protein diet, however, showed a retardation of growth at an early stage of the experiment and continued to grow at a somewhat sub-normal rate. At several periods during the experiment these two animals showed symptoms of joint trouble and lameness. These symptoms seemed to disappear when the supplement of shark liver oil was increased but no increase of the growth rate followed. The condition of these cats, apart from this lameness, was good although there was a tendency to excitability. Fig. 2 shows the growth curves of the cats. The experiments were continued for four months when one animal from the high protein group was killed for examination. The

¹ By an error the salt mixture was not incorporated in this diet for the first half of the experimental period. Little or no improvement occurred when the mistake was rectified and this fact taken together with the results of the rat experiments which were satisfactory from the outset leads us to conclude that the failure to grow was not due to deficiency of salts. The salts of the yeast extract probably made up any deficiencies of the meat.

second animal in this group was then given the normal mixed diet and immediately responded by a rapid increase in growth rate so that after another month it had regained the normal weight for its age. The tissues of the cat killed from the high protein group showed no abnormality beyond very marked beading of the ribs and the normal condition of the organs was confirmed by microscopical examination.

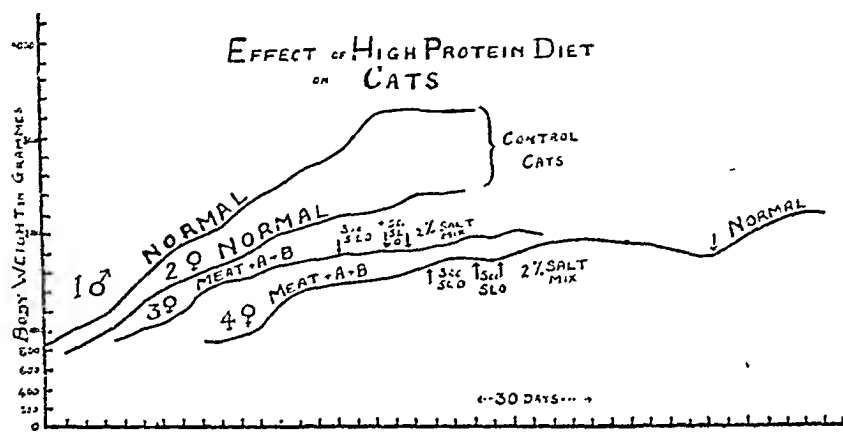


Fig. 2

Table III shows the relative weights of the animals throughout the experiment.

		Relative weights			
Diet		Start	1 month	2 months	3 months
Normal.	Male	1	2	2.9	3.5
	Female	1	1.9	2.5	2.9
Protein.	Female	1	1.7	1.9	2.1
	Female	1	1.7	1.9	2.1

Remarks. The failure of a typical carnivorous species to show normal growth on a high protein dietary supplemented with what were believed to be adequate amounts of inorganic salts and vitamins was surprising but curiously confirmative of the results with the rats. The normal condition of the organs in our experimental animals leads us to infer that Chalmers Watson's results were largely attributable to the fact that his dietaries were deficient in the accessory factors.

In our experiments on rats with a high protein diet, microscopical examination showed no change in the tissues. The kidneys remained normal in appearance although they excreted 1.5 to 2 grms. of nitrogen daily for long periods. Thus the statement that excretion of large amounts of nitrogen end-products causes degenerative changes in the kidney is not confirmed.

In her studies of lactation on high protein diets Miss Hartwell has, in her last paper⁽⁶⁾, to a certain extent considered the obvious criticisms of her earlier experiments and has taken some account of the possible influence of other dietary factors. She finds that the adult rat and the young growing rat can live normally on the diet containing an excess of protein (proportion 15 gm bread to 5 gm protein) but that the same ration is not adequate for normal milk production in the lactating female unless definite quantities of some other constituent or constituents are supplied. Before this paper appeared we commenced a series of experiments designed to throw light on her results and their bearing on our high protein feeding tests already described. These latter experiments are as yet incomplete but we have obtained definite evidence that a high protein ration (83 p c of dry weight of diet in the form of caseinogen) can be adequate as a diet for lactating female rats and enables them to rear healthy litters of normal weight provided that it is also adequate in components serving as sources of the vitamins. We have also found that the diet of the mother prior to pregnancy and lactation is a factor of fundamental importance and one to which Miss Hartwell may not as yet have given sufficient attention. Miss Hartwell's preliminary results with yeast extract, milk, butter etc, tend to show that a factor present in yeast extract and milk is protective. There is a certain amount of scattered evidence that vitamin B which is contained in yeast is directly or indirectly concerned quantitatively with the metabolism of carbohydrate (subject reviewed by Funk⁽⁷⁾) and if this is correct it is justifiable to assume that it will be equally concerned in the metabolism of the non nitrogenous fragments of the amino acids which normally undergo breakdown along the paths of carbohydrate metabolism. In this connection it is interesting to note that one of us has frequently observed the typical symptoms described by Hartwell in litters of rats but only in the case of those being reared by mothers on diets deficient in vitamin B. Her negative results with butter confirm our one observation that raising the quantity of shark liver oil did not restore the normal growth of the cat on the high protein ration.

SUMMARY

1. Growth, but at a sub normal rate, was shown by rats and cats fed on dietaries containing 80-90 p c of the dry weight in the form of protein (caseinogen) but apparently adequate as regards vitamins and salts. On changing to a normal diet the animals reach normal weight.

2. The rats maintained excellent health throughout but did not reproduce whilst on the special diet.

3. The organs and tissues presented a normal appearance at the end of the experiment and there is little doubt that the abnormal conditions found in similar experiments by other workers were due to causes other than the high protein intake, *e.g.* vitamin deficiency, etc.

4. The excretion of very large amounts of nitrogenous waste products over considerable periods of time does not appear to cause damage to the kidney in these species.

5. The failure to grow normally and to reproduce is in our opinion due not to the high protein intake itself but to a lack of balance between this constituent and some other component or components of a normal diet. It is tentatively suggested that the vitamin B, which is believed to be quantitatively concerned in the metabolism of carbohydrates, may likewise be concerned in the metabolism of those non-nitrogenous fragments of the amino-acids which follow a similar fate.

We are indebted to the Medical Research Council for a grant which defrayed the cost of these experiments.

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GASEOUS INTERCHANGE IN THE STOMACH IN THE ANÆSTHETISED ANIMAL. BY NORA EDKINS.

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THE object of the following investigation was to determine whether there is any secretion or absorption of CO_2 or O_2 in the stomach. Secretion of CO_2 has been asserted by Schierbeck⁽¹⁾ to occur in dogs, and according to his results the amount secreted varies with the secretory activity of the gastric glands. He washed out the stomach with water, freed from CO_2 by boiling, at a temperature of 37°C . and at intervals withdrew portions of the water and analysed them for CO_2 . In fasting animals he found the tension to be 30–40 mm. Hg. In order to observe the influence of activity he introduced food and water into the stomach, and during digestion found that the tension of CO_2 rose rapidly and at the height of digestion reached a pressure of 130–140 mm. Hg. It was obvious that by this method CO_2 might be formed by bacteria introduced with the food. More recently Ylppo⁽²⁾ has found that large quantities of O_2 and CO_2 are absorbed when introduced into the stomach of man; the absorption may, however, have taken place in the intestine.

Method. It is clearly of advantage in testing for any interchange of gas during activity to avoid the complication caused by the presence of food in the stomach. In my experiments I was able to achieve this by injecting gastrin intravenously whilst the stomach contained only introduced gas and normal saline.

The animals were taken fasting. After preliminary anæsthesia with chloroform and ether the animals (cats) were under the influence of urethane. The abdomen was opened, a ligature was tied round the œsophagus, an opening made in the duodenum just beyond the pylorus and a cannula ligatured through the pylorus into the stomach. Through this cannula recently boiled out warm saline was introduced for washing out the stomach cavity, and by careful manipulation all gas was expelled. A gas sampling tube, containing a known volume of gas of known composition, was attached to the pyloric cannula and the space between the stomach and the tube rendered gas free by withdrawing the remains of the washing out saline in the stomach through the side tube of the sampler. The animal in most of the experiments was kept in a bath at 37°C ., only the head and shoulders being uncovered by the liquid.

Gas was introduced into the stomach by means of a mercury reservoir attached to the gas sampler and after the desired interval it was removed in the same way. The gas thus withdrawn was then transferred to the burette of a Haldane gas analysis apparatus and left disconnected from the rest of the apparatus for one hour to cool before the volume was determined. It was found in some preliminary experiments that after a period of two hours the gas, itself free from CO_2 , introduced into the stomach, attained a certain percentage of CO_2 and this showed no increase as the result of more protracted exposure to the gastric mucous membrane.

In some experiments 25 c.c. of sterilised saline were introduced in addition to gas and both were later analysed for CO_2 , while the former was also analysed for HCl. A known volume of the liquid from the sampler was run under the surface of a known volume of baryta water and the excess of alkali titrated with standard oxalic acid. A similar volume of the liquid was then boiled carefully for five minutes to get rid of the CO_2 , treated with baryta and titrated with oxalic. The value of HCl in terms of the oxalic used was thus determined and the percentage of CO_2 and HCl calculated. In all these experiments control titrations of the sterilised saline employed for experiment were made. In a few cases sterilised saline only was introduced and the liquid withdrawn after an interval of two hours and analysed by the method just described. In experiments in which the gastric mucous membrane was rendered active by gastrin injections, both gas and saline were introduced. In all cases it was found that the tensions of CO_2 in both the gas and the liquid were identical.

The effect of manipulation of the stomach in removal of any solid matter present before the stomach had been washed, was investigated by giving the animal a meal of meat about an hour before anaesthetising it. In these cases there was found to be an increase in the CO_2 produced. The mucous membrane of the stomach was almost invariably examined for micro-organisms at the end of the experiment and in some cases obvious catarrhal changes were found to be present. These experiments were excluded. A certain number of experiments were performed to compare the tensions of CO_2 in the stomach and in alveolar air, taking as alveolar air the last portion of expired air. The tracheal tube was replaced by a T-tube of somewhat narrower bore with a two-way tap at the angle of the T-piece. One arm of the T-piece was connected to a gas sampler which was filled with Hg from a reservoir and Hg was brought up to the tap. The tap was turned so that the trachea was connected

with the an during inspiration and at the end of expiration it was rapidly turned on and off to the sampler, so that by having a slight negative pressure (about 1 cm Hg) in the sampler about 0.1 or 0.2 c.c. was collected at each breath. Repeating this for about 1-5 minutes, about 20-25 c.c. of alveolar air could be collected. Some practice was required before this method of collecting the ultimate expired air could be relied on and if the animal were breathing at all rapidly the difficulty of collecting in this way rendered the method impracticable. There was no difficulty up to a rate of 50 respirations per minute.

I *Introduction of 20 c.c. of N_2* . In these experiments the cat was fixed to an animal stage and the abdomen was kept warm by hot flannels repeatedly applied. In one hour a slight gain in volume was noticed. The percentage of CO_2 was about 7 and of O_2 about 0.65. In two hours the gain of total volume was slightly greater, the percentage of CO_2 averaged 10 and O_2 was generally higher than in one hour. In three hours the values obtained were of the same order as in two hours.

II *Introduction of 20 c.c. of a mixture of N_2 and O_2* . In these and subsequent experiments the standard period of exposure was two hours. Nine experiments were made which are illustrated in the sample experiment adduced.

Gas introduced 17.1 c.c. containing 2.27 p.c. O_2 and the remainder N_2 .

Volume on removal 17.95. Percentage of CO_2 in removed gas 9.7 and of O_2 1.39.

In all these experiments there was a large gain of CO_2 and a loss of O_2 .

In the preceding experiments, I and II, I do not regard the condition of the animals as having been normal. Though the temperature of the room was never low, usually about $17^\circ C$, it is probable that the heat loss was considerable and influenced the CO_2 exchange. In the rest of the experiments the animal was kept in a warm bath at $37^\circ C$.

III *Activity of gastric mucous membrane induced by injections of gastrin*. In these experiments 2 c.c. of a gastrin extract, neutralised immediately before use so that it had a pH of about 7.4, were injected into the femoral vein at intervals of 15 minutes for the first $1\frac{1}{4}$ hours of the experiment. To test the activity of the gastric mucous membrane as the result of these injections the method adopted was to introduce with pure N_2 about 20 c.c. of sterile normal saline. The gaseous interchange was estimated as before (the gas coming in contact with the anterior mucous membrane of the stomach without the intervention of the liquid) and the liquid subsequently removed, analysed for total acidity and acidity after boiling out the CO_2 as described previously.

The CO_2 percentage in the removed gas was lower and showed very much less tendency to vary than in the previous experiments. In three experiments in which gastrin was used, the CO_2 percentage was 7.2-7.9 (tension 51.3-56.3 mm. Hg), the O_2 about 1 and the HCl about 0.1. In the liquid the CO_2 tension was identical for the temperature with that in the gas mixture showing complete equilibrium between the two.

IV. *Animal fasting. N_2 and saline introduced.* In this series the gastric mucous membrane was at rest but though the time of exposure was frequently raised to three hours, the percentage of CO_2 varied between 5 and 5.5 and the O_2 between 0.75 and 1.4. The tension of CO_2 was identical both in the liquid and the gas mixture.

V. *Animal fasting. N_2 and O_2 , latter averaging about 1.6 introduced.* The CO_2 remained about the same as in the 4th series but the O_2 fell to 1 p.c. or less.

VI. *Animals in full digestion.* The animals were fed with minced meat an hour before the experiment was begun. At the commencement of the experiment the food was removed through the pylorus which involved sometimes considerable manipulation of the stomach walls. The experiments were designed to ascertain whether this manipulation affected the production of CO_2 . The CO_2 percentage averaged 6.5 p.c., but the question arises as to whether the higher percentage of CO_2 was due to the manipulation or to the fact that the stomach was in a condition of activity at the beginning of the experiment. Without further elucidation of this point there is no reason to believe that the increased production of CO_2 was due to other causes than the fact that the stomach was in a state of activity at the beginning of the experiment.

Observations were also made with a view of seeing whether the quiescent stomach evolved CO_2 . The stomach was washed with boiled-out saline to remove any gas present. The liquid was withdrawn and the stomach was left free of both gas and liquid for three hours. At the end of this period, gas-free saline was again introduced into the stomach for the purpose of washing out any accumulation of gas and removed as rapidly as possible. No gas was obtainable and the liquid on analysis was found to contain no appreciable CO_2 .

From this experiment it would seem that there is no recognisable secretion of CO_2 into the cavity of the stomach during the resting period. In contrast with this, experiments have been performed with liquid alone in the stomach and in these cases the liquid was found at the end of the experiment (two hours) to contain 3 p.c. of CO_2 , equivalent to a tension of 41.8 mm. Hg.

VII. *Introduction of acid saline and N_2 into stomach at rest.* These experiments were made to determine whether acid in the stomach could set free CO_2 from bicarbonates in the mucous membrane and thus account for the rise of CO_2 in secretory activity. This possibility was negatived. After two hours the CO_2 varied between 5.5 p.c. and 6.3 p.c. indicating that the rise in CO_2 during secretory activity is not due to the mere presence of HCl in the stomach reacting with carbonates or bicarbonates in the stomach wall.

VIII. *Alveolar air analysed at intervals throughout the experiment.* The alveolar air was collected at about half-hour intervals. In most cases gastrin was injected. In all cases it was found that the average percentage of CO_2 in alveolar air throughout the experiment was about 0.3–1.1 p.c. lower than that found in the stomach. In other words, the pressure of CO_2 in the tissue of the stomach was greater than that in alveolar air (ultimate expired air).

CONCLUSIONS AND SUMMARY.

A gas mixture was introduced into the stomach cavity and restricted to it.

It is unnecessary to explain the presence of CO_2 in the stomach as due to the active secretion of this gas by the gastric mucous membrane. When the stomach is washed out there is no accumulation of CO_2 even after an interval of three hours. When N_2 , N_2 and O_2 , or N_2 and saline, were introduced into the fasting stomach, the CO_2 reached a fairly constant level. I conclude that the presence of CO_2 is due to passive diffusion from the tissues of the wall of the stomach into the cavity.

With the stomach at rest the tension of CO_2 varies between 5.5 p.c. and 6.5 p.c. of an atmosphere = 39.93 mm. Hg and 46.3 mm. Hg. During activity in the absence of food the CO_2 rose to about 7.5 p.c. = 53.5 mm. Hg and the O_2 remained about 1 p.c.

Since alveolar CO_2 pressure was always found to be lower than gastric CO_2 pressure and O_2 pressure very much greater, it seems permissible to regard these experiments as comparable to the use of a tonometer for the purpose of gauging the tension of CO_2 and O_2 in the gastric mucous membrane. The conclusion may be drawn that the tension of CO_2 in this tissue is greater than that of venous blood and that the tension of O_2 is of a very low order.

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THE REFRACTORY PHASE IN A REFLEX ARC.

BY E. D. ADRIAN AND J. M. D. OLMSTED.

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IN the muscle-nerve preparation one of the phenomena which can be most accurately investigated is the refractory state following the passage of an impulse and the process of recovery therefrom. In the reflex arc many indications of a similar refractory state have been recognised; Sherrington and Sowton(1) in particular have shown that there is a minimal "interval for muscular summation" in the reflex arc just as there is in a muscle nerve preparation, since two stimuli applied to the afferent nerve will not give a summated contraction unless there is a definite time interval between them. It appeared to us that a general investigation of this phenomenon might be of value, and as a first step we were anxious to see whether the blocking of the reflex arc by anaesthetics would be accompanied by any increase in the interval for muscular summation.

In view of what happens in a frog's muscle nerve preparation a large increase might have been expected. In the sciatic gastrocnemius preparation the interval rises from about $\cdot 003$ sec. to $\cdot 02$ sec. when a narcotic such as alcohol is applied to a portion of the nerve between the electrodes and the muscle(2). This increase is not due to any slowing of the recovery process; with alcohol at any rate Lucas(3) has shown that the rate of recovery is unaltered. It depends solely on the fact that recovery is a gradual affair and that in the early stages of recovery the nerve is capable of transmitting only a very small impulse. Under ordinary conditions such an impulse can reach the muscle and give a summated contraction, but during the narcosis the affected region will conduct with a decrement and all impulses below a certain size will be extinguished. The interval for muscular summation must be increased, therefore, so that the second impulse may be large enough to pass the block. Eventually the block will become so great that only impulses of full size will be able to pass through and the interval for muscular summation will then be about $\cdot 02$ sec., since at this time the relative refractory phase is over and the second impulse is equal in size to the first. Any further increase in the narcosis will cause the first impulse to fail as well

as the second, and therefore the interval for summation will never rise above $\cdot 02$ sec. unless the anæsthetic produces an actual slowing in the rate of recovery.

In the motor nerve of a mammal the recovery process takes place at body temperature more rapidly than in the frog's nerves, but the interval for muscular summation is increased in the same way by local narcosis. This may be seen from Fig. 1 which records the effect in the muscle nerve preparation of a spinal cat. The motor nerve (peroneal) was cut through high up in the thigh and shielded stimulating electrodes were placed on it. Between the electrodes and the muscle (tibialis anticus)

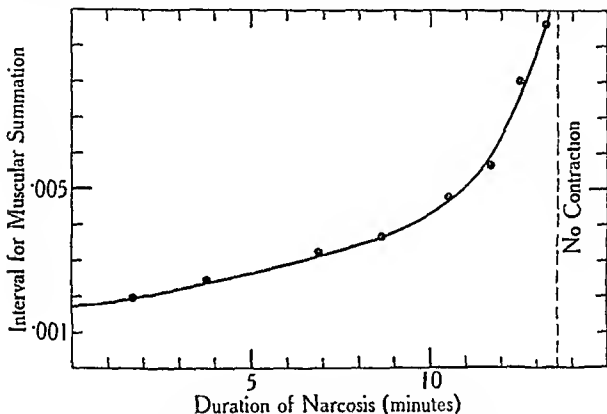


Fig. 1. Peroneal nerve and tibialis anticus muscle of spinal cat. Effect of local narcosis on the least interval for muscular summation.

the nerve passed through a vulcanite chamber 11 mm. in diameter in which 7 p.c. alcohol solution could be placed. The interval for muscular summation was recorded in the usual way on a stationary drum and its gradual increase is shown in the figure. It will be seen that just before conduction fails the interval is $\cdot 0095$ sec. At this stage the first impulse must be nearly extinguished in the narcotised area, so that the second impulse must be nearly as large as the first if it too is to escape extinction. We should therefore expect that the nerve takes just over $\cdot 0095$ sec. to return to its normal resting state after the passage of an impulse. This estimate agrees very well with the "recovery curve" which gives the rate of recovery of excitability in the cat's peroneal after the passage of

an impulse. Fig. 2 is a typical recovery curve from a similar preparation and it shows that the excitability has returned to 90 p.c. of its normal value in $\cdot 0085$ sec. Thus the two figures taken together show the effect of local narcosis and its explanation in terms of the gradual return of function after the passage of an impulse.

The same reasoning should apply to the passage of two impulses through a reflex arc. If a small second impulse travelling in incompletely recovered tissue is able to pass through the arc under normal conditions, the effect of an anæsthetic applied to the central part of the arc should be to prevent such an impulse from passing before it would one of full size; the interval for muscular summation should in-

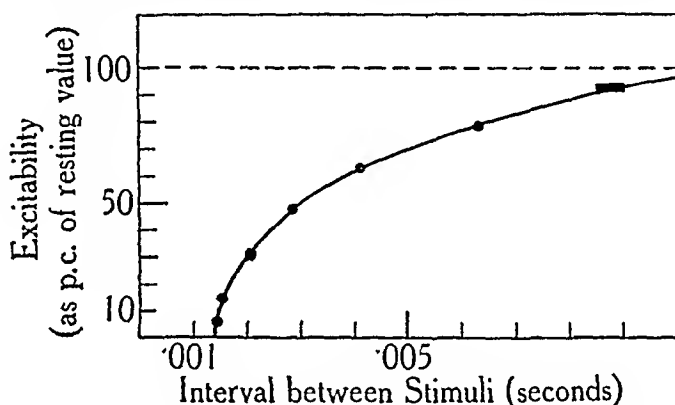


Fig. 2. Recovery of excitability in peroneal nerve of spinal cat after the passage of an impulse.

crease therefore and the final value just before conduction fails should be such that the second impulse when it enters the decremental region is travelling in tissue which has had time to recover completely from the passage of the first. In the flexion reflex arc of the spinal cat Sherrington and Sowton found that if two stimuli were applied to the afferent side of the arc the least interval for muscular summation was about $\cdot 001$ sec. This is only slightly greater than the interval required when the stimuli were applied to the motor nerve and, unless the sensory nerve differs considerably from the motor, it is not at all likely that the sensory nerve can have recovered completely in so short a time. Thus we might well expect to find a considerable increase in the interval for reflex muscular summation when the central nervous system is anæsthetised and the final value should give us the time of recovery of the central part of the arc.

^{P.}₁₁ The interval for muscular summation in the reflex arc. The point was

tested in five experiments on spinal cats. The animals were decapitated under deep anæsthesia; the anæsthetic was then discontinued and the preparation ventilated artificially. The nerves to the hamstrings and the anterior crural nerve were cut and the popliteal branch of the sciatic was separated from the peroneal and divided at the level of the knee joint. Shielded stimulating electrodes were applied to the central end of the popliteal and the reflex contraction of the tibialis anticus muscle was recorded on a stationary drum. The stimuli were break shocks from two induction coils without iron cores. The interval between the two stimuli was controlled by connecting their primary circuits with the two keys of a Lucas pendulum and their strength was adjusted by changing the resistance in the primary circuit. Both coils were connected to the same pair of electrodes. After the experiments on the reflex arc the peroneal division of the sciatic was cut through high up in the thigh and the stimulating electrodes were applied to it in order to determine the interval for summation in the efferent part of the arc.

As Sherrington and Sowton have pointed out, it is more difficult to obtain an exact value for the interval when the stimuli are on the afferent side of the arc, since the response is more variable than the myoneural twitch and more dependent on the strength of the stimulus. We found, however, that a fairly constant summation interval could be obtained by using both stimuli about 5-6 times the threshold strength, and this interval was not appreciably reduced by increasing the strength of the second stimulus. The values obtained in eight preparations (including the five on which the anæsthetic effect was tested) are given in Table I: they vary from .0012 sec. to .0024 sec. The intervals obtained by stimulating the motor nerve are usually shorter though the difference may be very slight. The values we have obtained for both reflex and motor nerve stimulation are appreciably longer than those of Sherrington and Sowton, who give .001 sec. for the reflex and .0007 sec. for the motor nerve. Possibly the limb was at a lower temperature in our experiments though the usual precautions were taken to prevent cooling. The second column gives the least interval for muscular summation during the period of anæsthesia just before the reflex response became too small to give any visible record on the drum. The anæsthetic was a chloroform and ether mixture the vapour of which was allowed to enter the artificial respiration system. The percentage was adjusted so that the reflex response to strong stimuli disappeared in about ten minutes; at this stage the anæsthetic effect was confined to the central nervous system, for in a control experiment the threshold of

of a delayed arrival of the second response when the two stimuli are separated by a very short time interval.

The maximum frequency of response in the reflex arc. Any delay in the conduction of an impulse which travels in the wake of another will have a noticeable effect when we determine the response of the arc to a series of stimuli instead of to two only. Suppose, for instance, that two impulses can just be set up in the sensory nerve at an interval of $\cdot 002$ sec. apart and that, owing to the delay of the second impulse they are $\cdot 004$ sec. apart when they reach the central part of the arc. Probably, then, we cannot make impulses arrive at the central part of the arc at intervals of less than $\cdot 004$ sec., and therefore we cannot produce a series of reflex responses less than $\cdot 004$ sec. apart however rapidly we may stimulate the afferent nerve. The maximum frequency of response is therefore 250 a second although the least interval for muscular summation is only $\frac{1}{500}$ of a second. It is true that many other causes besides delay might produce such a discrepancy; for instance, the recovery process might take place more slowly during the repeated stimulation; but if the discrepancy is not found we may safely conclude that the delay is negligible.

In the frog's muscle-nerve preparation, where a delay of the second impulse is known to occur, the maximum frequency of response is considerably slower than would be expected from the published figures for the least interval for muscular summation. This has been pointed out by Hoffmann(8) and by Beritoff(9); Hoffmann attributes it to the delay and Beritoff considers this a possible cause, though not the most important. In the case of the reflex arc, however, extensive investigations have been made by Beritoff, Foà(10) and others on the maximum frequency of reflex response in the frog's muscles, but their results do not help us since there are no measurements of the least interval for reflex muscular summation in the frog with which they may be compared. We have therefore determined the maximum frequency of response in the cat's tibialis anticus muscle to reflex and motor nerve stimulation and we have compared this with the least interval for muscular summation in the same animal.

For repeated stimulation with different frequencies we have used break shocks from an induction coil connected with a rotating contact breaker of the drum and segment type. This made and broke the primary circuit and short-circuited the make shocks sixteen times in each revolution. The shaft of the contact breaker was coupled direct or through a reduction gear to an electric motor; the speed was controlled by a

rheostat and a friction break and could be read at any moment by a speedometer of the magnetic type. String galvanometer records of the currents produced at different speeds showed that the apparatus would deliver a regular series of stimuli at any frequency from 16 to about 500 a second and that the speedometer gave a value for the frequency which was accurate to about 5 p.c. At higher speeds than 500 a second the galvanometer excursions often showed periodic variations in height though none of the stimuli were actually missed at the maximum frequency of which the machine was capable (800 a second). Erlanger and Garrey(11) have pointed out the danger of an interference of make and break induction effects with very rapid series of stimuli. To some extent the use of a coreless induction coil will minimise this difficulty since the duration of the make shocks will be very short, but the difficulty does not really arise in the present experiments as there was no need to keep the strength of the stimuli constant provided that all were strong enough to excite.

The frequency of response in the muscle was determined by photographic records of the action current made with the string galvanometer on cinematograph film. Arrangements were made for connecting the galvanometer through appropriate shunts with the stimulating circuit so that a record of the stimuli could be taken immediately after the record of the electric response without altering the string tension or the speed of the film. This made it possible to check the speedometer readings and to see at once whether the frequency of response was equal to or less than the frequency of stimulation. The speed of the film was recorded by a "phonic wheel" time marker which produced short lines .02 sec. apart at the edge of the film where they would not interfere with the action current records.

Five experiments were made on these lines. The spinal preparation was made as before and non-polarisable electrodes were attached to the tibialis anticus for connection with the string galvanometer. The stimulating electrodes were attached first to the afferent nerve (the popliteal branch of the sciatic) and the least interval for muscular summation was determined with two stimuli delivered by a Lucas pendulum. The electrodes were then connected with the rotating contact-breaker and a series of stimuli were delivered with frequencies ranging from 50 to 500 a second. The strength of stimulus was varied from about 2 to 20 times the threshold strength for a single shock. Several records were usually made with frequencies in the neighbourhood of 160 a second as this appeared to be near the critical value for the reflex arc, and these fre-

quencies were tested with several strengths of stimulus. In four out of the five experiments after the measurements on the reflex arc the efferent nerve (peroneal) was cut through high up in the thigh and the stimulating electrodes were transferred to it; the least interval for muscular summation was determined again and the stimulation with different frequencies was repeated.

Typical records of the response to various frequencies are shown in Fig. 4. At the slower rates the frequencies of stimulus and response

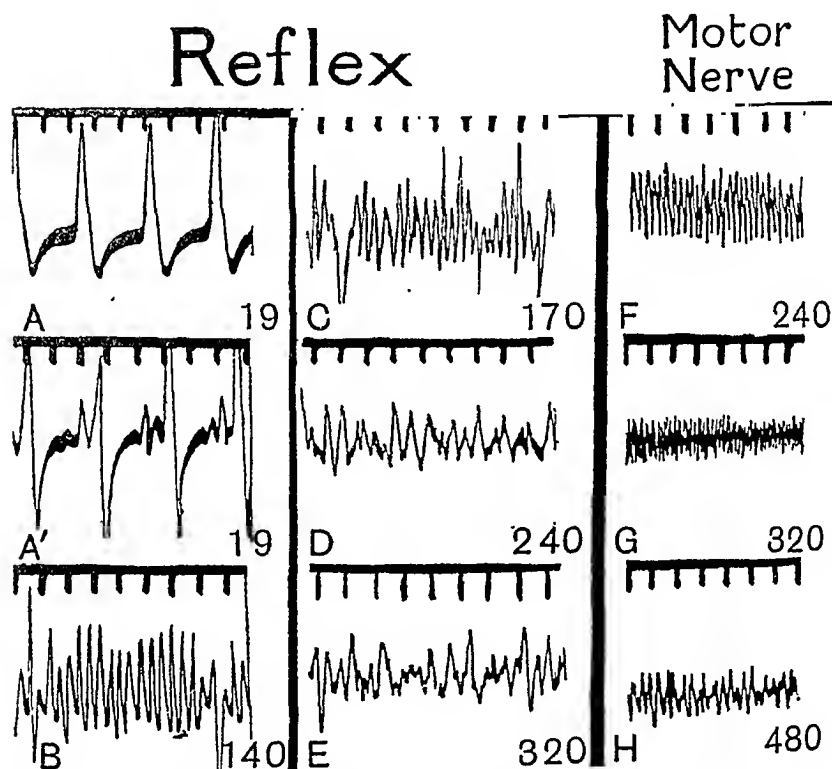


Fig. 4. Tracings from photographs of electric response of tibialis anticus stimulated by repeated shocks to sensory or motor nerve (Exp. 5).

A—E. Reflex.

- | | | | |
|-----|-------------------|-------------------------------|---|
| A. | 19 stim. per sec. | 3.5 times threshold strength. | Single response to each stimulus. |
| A'. | 19 " | 68 times threshold strength. | Multiple responses. |
| B. | 140 " | " | Responses at same frequency. |
| C. | 170 " | " | Responses at same frequency. |
| D. | 240 " | " | Responses irregular, about 160 per sec. |
| E. | 320 " | " | Responses irregular, about 140 per sec. |

F—H. Motor nerve stimulation.

- | | | |
|----|--------------------|---|
| F. | 240 stim. per sec. | Responses at same frequency. |
| G. | 320 " | Responses at same frequency. |
| H. | 480 " | Responses irregular, about 220 per sec. |

Time marker gives .02 sec.

agree, each stimulus giving rise to one response and one only. In this our results differ from those of Beritoff who found that in the frog each stimulus to the sensory nerve would give rise to several responses in the muscle unless the frequency of stimulation exceeded 100 a second. With the spinal cat we have found that each stimulus produces multiple responses when a very strong current is used but not otherwise. This agrees with the work of Forbes and Adrian⁽¹²⁾ on the response to single break shocks in the flexion reflex of the spinal cat. With frequencies above 100 a second the height of the responses varies irregularly but the rhythm is still followed up to about 140 a second for reflex and 320 for motor nerve stimulation. Rhythms of 200 a second for the reflex and 400 for the motor nerve are sometimes followed for a few hundredths of a second at the beginning of a series but such rates are never maintained.

The results of all the experiments agree closely. Their essential features are shown in Table II which gives the maximum frequency of

TABLE II. A. Stimuli to sensory nerve

Exp.	Maximum frequency of response per sec.	Least interval between successive responses sec.	Interval for muscular summation sec.
4.	135 + 160 -	0074	0024 + 0020 -
5.	170 + 240 -	0059	0020 + 0016 -
6.	155 + 225 -	0065	0018 + 0016 -
7.	160 + 200 -	00625	0016 + 0014 -
8.	125 + 160 -	0080	0020 + 0016 -
Average	149	0068	0019

B. Stimuli to motor nerve.

4.	—	—	0014 + 0012 -
5.	320 + 400 -	0031	0018 + 0016 -
6.	320 + 400 -	0031	0018 + 0016 -
7.	320 + 400 -	0031	0016 + 0014 -
8.	315 + 400 -	0032	0018 + 0016 -
Average	317	0031	0017

response with stimuli applied to the sensory and to the motor nerve together with the corresponding intervals for muscular summation. The

"maximum frequency" referred to in the table is the maximum frequency which can be maintained for half a second at a time. From this it is easy to calculate the least interval between successive responses and these are given for comparison with the least interval between two stimuli necessary for muscular summation. It is clear that in the reflex arc there is a very great difference between these two intervals. The interval between the successive responses to a series of stimuli cannot be made smaller than .0059--0080 second whereas two stimuli alone will give summation (*i.e.* will give two responses) although they are only separated by .0016--0024 second. The difference is still present but much less in amount when the stimuli are applied to the motor nerve.

These results might well be due to a delayed appearance of the second of two impulses in the effector side of the reflex arc, as we have suggested above. But they are not conclusive evidence on this point since the limiting frequency with serial stimulation might be determined by some other factor which does not come into play when only two stimuli are used. It appeared to us that the question could not be settled conclusively without a direct comparison of the interval separating two responses and the interval separating the stimuli which gave rise to them. We have therefore made records of the electric responses to two stimuli for comparison with the records of serial stimulation.

Measurement of the delay of the second response. In these experiments we have used the capillary electrometer to record the action currents of the muscle in response to two stimuli on the afferent or efferent side of the arc. A recent investigation (13) of the accuracy to be expected from analysed electrometer records showed that the probable error in time measurements was much less than the error we should expect from uncorrected string galvanometer records. The capillary electrometer is, of course, not nearly so sensitive as the string galvanometer, but this is no drawback when the response of a large muscle is to be measured. The excursions of the mercury were photographed on plates travelling at about one metre per second and we found no difficulty in obtaining records suitable for analysis.

The experiments were carried out as follows. The reflex preparation was set up as before with stimulating electrodes on the afferent nerve (popliteal) and leads from the tibialis anticus muscle. The least interval for muscular summation was determined and electrometer records were made of the responses to two stimuli separated by intervals ranging from .002 to .01 second. Records of the response to repeated stimulation at different frequencies were then made with the string galvanometer, this

instrument being used because it had a more convenient recording apparatus for the purpose. After these measurements on the reflex arc, the sciatic was cut high up in the thigh, the electrodes were transferred to the efferent (peroneal) nerve and a similar series of records were taken with double and repeated stimulation. The full procedure was carried out in two experiments (Exps. 7 and 8) and the reflex measurements only in a third (Exp. 6). The results of these have already been stated in part in Table II. The results as regards the delay of the second impulse are strikingly concordant. In all of them with reflex stimulation the least interval between the two electric responses is very much greater than the least interval between two successful stimuli; in other words, there is a considerable delay in the appearance of the second electric response in the muscle when the two stimuli are separated by a short time interval. The delay is still present but very much smaller when the stimuli are

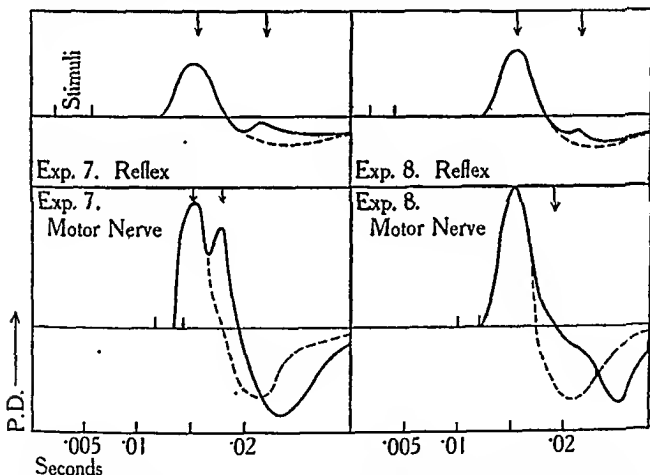


Fig. 5. Corrected capillary electrometer records of tibialis anticus response to two stimuli separated by a very short time interval. With reflex stimulation the delay of the second response is much greater than with stimulation of the motor nerve.

sent into the motor nerve. The existence of the delay and the difference between the reflex and motor nerve effect may be seen from Fig. 5 which gives some analysed electrometer curves from two experiments. In

these curves the dotted line represents the continuation of the first electric response as determined from records with one stimulus only. The arrows show the moments at which the two responses reach their maximum and this probably gives the best measure of the interval between them, since the beginning of the responses is not so easily determined. The figures show the characteristic differences in the latent periods with reflex and motor nerve stimulation as well as the much greater delay of the second response in the reflex.

The extent of the delay in relation to the interval between the two stimuli may be seen from Fig. 6 which records the most complete series of measurements on the reflex arc (A) and on the muscle nerve preparation (B). The two series are from different animals since the corresponding measurements from one and the same animal would not cover such a wide range. The figure is constructed on much the same lines as are those given by Lucas (5) in his paper on the delay phenomena in the tissues of the frog. A comparison with his curves shows no essential

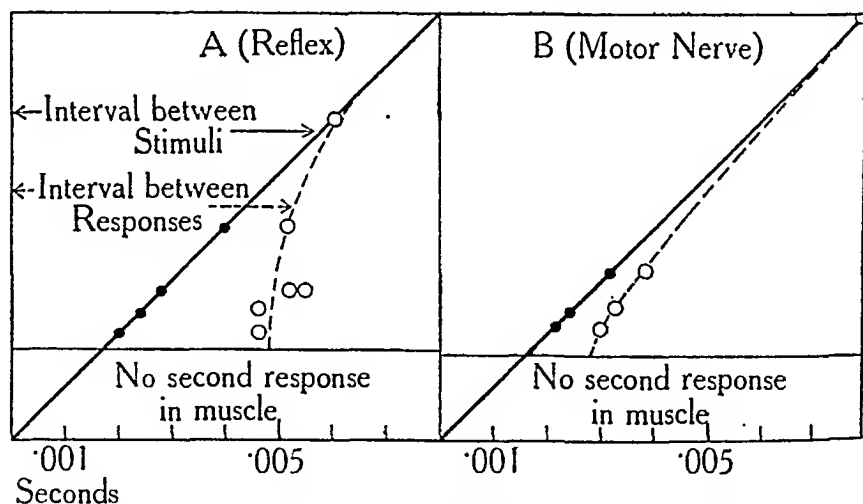


Fig. 6. Relation between the delay of the second response and the interval between the two stimuli.

difference in the phenomenon though the delay is considerably smaller in the mammalian muscle nerve preparation than in that of the frog.

These results show that if two stimuli separated by a very short time interval are applied to the afferent nerve of the arc, the second impulse appears in the muscle much later than it would have done if the stimuli had been applied to the efferent nerve instead. Thus a considerable delay occurs somewhere in the afferent side of the arc and this delay must be

taken into account before we can make an estimate of the rate of recovery of the synapse. Before we attempt this, however, it is interesting to consider whether the delay we have found is enough to account for the great difference in the least interval for reflex muscular summation and the least interval between successive reflex responses. The essential figures are set out in Table III. This gives the capillary electrometer

TABLE III. A. Stimuli to sensory nerve

Exp	Least interval for muscular summation (two stimuli) sec.	Interval between 1st and 2nd response sec.	Least interval be- tween successive responses sec.
6.	·0018	·0046	·0065
7.	·0016	·0064	·00625
8.	·0020	·0057	·0080

B Stimuli to motor nerve.

7.	·0016	·0024	·0031
8.	·0018	·0033	·0032

measurements of the least interval between two isolated responses and also the least interval between the successive responses in a series. In two out of three experiments the interval is longer when the responses form part of a series, but the difference is quite small. The state of affairs

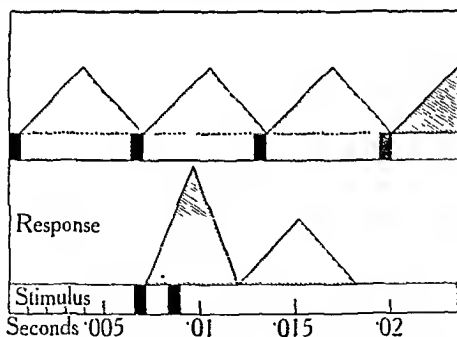


Fig. 7. Intervals between stimuli and between responses with reflex stimulation (Exp. 6). Upper half shows repeated stimulation at rhythm giving maximum frequency of response. Lower half shows two stimuli at least interval for muscular summation.

revealed by this table is shown more clearly by Fig. 7 which gives the intervals between stimuli and responses in Exp. 6. In the upper half of

the figure a series of stimuli and responses are shown occurring at the maximum rate which the reflex arc can follow, *i.e.* 155 a second; for simplicity the responses are drawn as though they occurred immediately after each stimulus. The lower half shows two stimuli separated by the least interval for muscular summation ($\cdot 0018$ sec.) and the two responses corresponding to these stimuli separated by a much longer interval ($\cdot 0046$ sec.). It will be evident from this part of the diagram that although two isolated stimuli $\cdot 0018$ sec. apart can give two responses in the muscle, yet, owing to the tardy appearance of the second response, the least interval between the successive responses in a series cannot well be less than $\cdot 0046$ sec. Actually it turns out to be $\cdot 0065$ sec. when the series lasts a second or more. This may be due to an increase in the delay with repeated stimulation or to some other cause, but in any case the delay seems to be much the most important factor in determining the maximum frequency of response in the arc. The same holds good for the frequency of response in the muscle nerve preparation. The second part of Table III gives the figures for the two experiments in which the stimuli were sent into the motor nerve and it will be seen that the least interval between two isolated responses agrees very closely with the least interval between the successive responses in a series, although the interval for muscular summation is shorter.

These results give a satisfactory explanation of the great discrepancy between the least interval for muscular summation and the maximum frequency of response in the reflex arc. The cause of the delay is still unexplained. We have not attempted to deal with it in the present research and there are not enough data even to say whether it occurs in the setting up of the impulse or in its conduction.

Rate of recovery in the reflex arc. We are now in a position to estimate the maximum time which the central part of the arc must take to recover its normal conductivity after the passage of an impulse. The experiments in the last section show that the least interval which can elapse between the arrival of two impulses in the central part of the arc is about $\cdot 006$ sec. The experiments with anæsthetics showed that the second of two impulses separated by the least possible interval has just as much chance of passing through the anæsthetised region as had the first impulse. This means that the region must have recovered its normal conductivity by the time the second impulse arrives, and the maximum time which it can take for the recovery to normal is therefore $\cdot 006$ sec.

An objection may be brought against this conclusion on the ground that the figure $\cdot 006$ sec. gives the least possible interval between two

impulses in the arc under normal conditions. Our argument assumes that this interval does not change when conduction in the arc has been nearly abolished by an anæsthetic. It is quite conceivable that the interval would then become longer, and if it did so our estimate for the time for recovery would have to be increased. To settle this point we have determined the maximum frequency of response in the arc before and during a period of anæsthesia. This was done in two experiments and in neither was there any appreciable change in the maximum frequency although the response was nearly abolished by the anæsthetic. Thus the least interval between successive responses is not altered by anæsthetising the central part of the arc and our estimate of $\cdot 006$ sec. for the maximum recovery time may be allowed to stand.

In considering this result it must be remembered that we cannot be sure that the synaptic region, or region affected by anæsthetics, takes any time at all to recover. We have no direct evidence that it enters into a refractory state after the passage of an impulse, for the existence of a minimal interval for reflex muscular summation may be due to a refractory state either in the sensory nerve or in some part of the central nervous system other than the synaptic region. The latter may be able to remain continuously in the excited state without the periodic intermissions which must occur in the peripheral parts of the arc. This view is not in harmony with the idea that the process of conduction is much the same in the central parts of the arc as in the peripheral, nor does it agree with the conclusions of Fröhlich(14), Veszi(15), Tiedermann(16) and others who have worked on the refractory phase in the spinal frog. In the present case the delay of the second impulse indicates some alteration in conduction which it is natural to associate with imperfect recovery, though it is quite conceivable that the delay occurs in a different region from that in which the anæsthetic block takes place. The absence of any refractory phase in the synaptic area is therefore a possibility which must be taken into consideration, though it need not deter us from investigating the consequences which would follow if the area passed through the usual absolute and relative refractory stages after the passage of an impulse.

The estimate of $\cdot 006$ sec. for the maximum time for recovery applies only to that part of the arc which is affected by the anæsthetic; parts which are not affected may recover more slowly, and indeed we have already seen that the motor nerve takes $\cdot 01$ sec. for recovery to normal. The synaptic area may recover in less than $\cdot 006$ sec. but it is unnecessary to suppose that it takes much less than this. Forbes and Gregg(17)

have suggested that some part of the synaptic region must recover very rapidly since it is possible for two impulses to produce a reflex effect where one fails, although the two impulses have been set up very close together in the afferent nerve. For this kind of summation we should expect that the second impulse would have to arrive during the "supernormal phase" of recovery in the synaptic region, *i.e.* after the recovery to normal is over. But we have seen that if two impulses come through the arc they are spaced out to $\cdot 006$ sec. apart however short the interval between the two stimuli which set them up. Consequently the second impulse might arrive during the supernormal phase of recovery although the recovery to normal took nearly $\cdot 006$ sec.

It is in fact an interesting consequence of the delay effect that impulses can only come through the synaptic region at intervals of $\cdot 006$ sec. at which time recovery to normal is always complete. This may possibly have some bearing on the difference between inhibitory and excitatory paths in the central nervous system, for it would be impossible to obtain the Wedensky type of inhibition under these conditions. Further deductions would be of little value in the absence of other quantitative measurements and of some further knowledge of the factors which produce the delay of the second response. For this reason we have avoided discussing the present results in connection with the scheme of central connections outlined by Forbes (18). The experiments seem to us of interest as offering a method of general applicability for measuring the refractory phase phenomena in different arcs. It is true that the measurements are none too straightforward but the results are quantitative and directly comparable with those obtained from simple conducting tissues.

CONCLUSIONS.

1. In the arc for the flexion reflex in the spinal cat we find that the least interval which must separate two stimuli giving a summated contraction is about $\cdot 0019$ sec. when the stimuli are applied to the afferent nerve and $\cdot 0017$ sec. when they are applied to the efferent nerve. These values are slightly longer than those given by Sherrington and Sowton.

2. When the arc is stimulated by repeated shocks at different frequencies the maximum frequency to which the muscle will respond synchronously is on the average 150 a second for stimulation of the afferent nerve and 320 a second for the efferent. The least interval

between successive responses is therefore $\cdot 0067$ sec. for afferent nerve stimulation and $\cdot 0031$ sec. for efferent.

3 With afferent nerve stimulation the discrepancy between the least interval for muscular summation and the least interval between successive responses depends on the fact that if only two stimuli are used the second response does not appear in the muscle until 005-006 sec after the first, however close together the stimuli may have been. The delay of the second response occurs in the afferent nerve or in the central part of the arc. With efferent nerve stimulation the second response is delayed until 003 sec after the first.

4 When conduction in the central part of the arc is abolished by anæsthetic the interval for reflex muscular summation remains constant until conduction fails. Thus the second impulse, which arrives at the anæsthetised region 006 sec after the first, has no greater difficulty in passing through than had the first impulse. The region on which the anæsthetic takes effect ("the synaptic region") has therefore recovered its normal conductivity within 006 sec of the passage of an impulse.

5 The recovery process in the synaptic region is shorter than in the motor nerve (which recovers normal conductivity in 01 sec). There is, however, no reason to suppose that recovery takes much less than 006 sec in the synaptic region though it may conceivably do so.

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THE RENAL FUNCTION AS JUDGED BY THE EXCRETION OF VITAL DYE-STUFFS. BY J. DE HAAN.

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Holland.)

IN recent years the evidence for the secretion of specific substances (especially dye-stuffs) by the kidney cells has been subjected to much criticism. The earlier conclusions drawn from them were, in fact, drawn without knowledge of various important properties of the substances used. Schulemann⁽¹⁾ from an investigation of hundreds of dyes showed that the rate of their diffusion through the animal body closely resembled that of their diffusion *in vitro*, so that in one case, as in the other, their diffusion is directly dependent upon the size and the more or less colloidal properties of the dye-stuff molecule. Von Möllendorff⁽²⁾ in a systematic research found a definite relation between the colloidal properties of dyes and the rate at which they are secreted by the kidneys. His observations have not had the recognition they deserve; they are not mentioned by Cushny⁽³⁾ in his discussion of the secretion of dyes. Perhaps the most convincing of von Möllendorff's experiments is that of the renal behaviour towards injected trypan blue. The more diffusible portion of the dye is secreted first, then follows the blue stain which is the chief ingredient, in perfect conformance with the degree of diffusibility *in vitro*. The dye is seen first as a granular staining in the cells of the convoluted tubules, the intensity decreasing in the direction away from the glomerulus; but it does not become visible, or only slightly, until secretion has reached its highest point. The maximal concentration of the dye in the cells appears much later and persists for days when secretion of the dye has practically ceased. Thus in all probability the presence of the dye in the tubule cells has nothing to do with its secretion by these cells. The histological appearance I may add can be readily produced by injecting 0.25-0.5 c.c. of 1 p.c. trypan blue into a mouse. It is almost identical with that described by Suzuki⁽⁴⁾ after injection of lithium-carmines.

Based on his collective results, von Möllendorff concludes, and rightly, I believe, that the picture of dye secretion points to a localised elimination of dyes in a diluted form by the glomerulus, according to

physico-chemical laws. It will be readily admitted that the glomerular membrane which has practically no thickness is better fitted for permeation by dissolved substances than the tubular cells with their complex structure.

If then we hold the primary glomerulus filtrate to contain all the secreted dye in a very dilute form, we must assume a high degree of inspissation by reabsorption of water and of some dissolved substances, to account for the much higher concentration of the same dye in the urine. Cushny has thrown light on the degree of concentration for example with regard to urea, SO_4 , etc. and he assumes that urea is not appreciably reabsorbed by the tubules. He has pointed out that, on the ground of the urea concentration in some animals as cats, there must sometimes take place in the tubules an inspissation equal to 100. Now, I attempted to determine by experiments if in this respect urea and acid dyes behave in the same manner, in other words, I wanted to find out whether urea and dye-stuffs were not appreciably reabsorbed. If so, the relation between the concentration of urea and injected dyes, present in the urine and in the blood plasma, would have to be the same at the same moment. On the ground of earlier experiments (5) I had to consider the fact that the permeability of the glomerular membrane to acid dyes must be influenced by another phenomenon: the fact of these dyes being almost entirely attached to the plasma colloids by adsorption.

Thus it could be shown that the very diffusible substance fluorescein potassium, dissolved in serum, passes only in very small quantity through the membrane of an ultra filter, whereas in watery solution it goes through entirely. Furthermore, the behaviour of fluorescein potassium *in vitro* resembled greatly certain phenomena after injection of that substance *in vivo*. Thus in a fluid, free from albumen, like the aqueous fluid of the anterior chamber of the eye, the dye after intra abdominal injection, reached a disproportionately lower concentration than in the blood serum. Further experiments showed the same adsorption to exist for many, if not all, other acid dyes.

Now, this fact had an important bearing on the renal problem in connection with the rather generally accepted hypothesis, also assumed by Cushny, that the glomerulus membrane is practically impermeable to colloids. Like the aqueous fluid of the anterior chamber, the glomerulus filtrate can on this view contain dye-stuff only at a very low concentration, greatly inferior to that in the plasma, at the most at the concentration of that part of the dye which is not adsorbed in the plasma of the blood.

From what I had observed regarding the concentration of dyes as fluorescein and trypan blue in the urine, it seemed probable that we should have to assume an extraordinarily great secondary inspissation of the glomerulus filtrate in substances like trypan blue, a concentration so incredibly high as to make the axioma of an albumen free glomerulus filtrate rather doubtful.

Assuming the glomerulus filtrate to be free from protein and no appreciable amount of dye-stuff to be reabsorbed, I have tried to determine the degree of concentration of the various dyes, that would have to be assumed during the formation of urine and to find out simultaneously the difference between the concentration of urea in the urine and in the blood plasma. Adsorption of the urea to the plasma colloids needs, as is well known, not to be considered. This test would also decide whether as Cushny holds urea is not reabsorbed.

My method of experimentation was as follows:

The animals used were rabbits, since it takes little trouble to get a desired quantity of blood from the ear. The bladder, preferably of male animals, was emptied by catheter and immediately after a rather large quantity (100–150 mg.) of dye was intraperitoneally injected. In one case, when highly colloid substances were used, the injection was made directly into the blood, since we know that in these cases the dye loses but little of its concentration during the time, sufficient to collect the quantity of urine needed. Working with more diffusible dyes, it was better to inject outside the circulation to obtain a somewhat constant concentration in the blood for one or two hours. At different moments after injection 1 c.c. of blood was collected into a graduated centrifugal tube, containing 3 c.c. of 0.7 p.c. NaCl solution and 1.1 p.c. of sodium-citrate. After centrifugalisation the dye concentration was determined, taking into account the dilution by the citrate and the volume of the blood corpuscles. In this manner it could be stated that the dye concentration in the plasma remained about the same for a sufficient time after the injection.

A larger quantity of blood (4.5 c.c.) was taken both immediately before the injection of dye, and at the end of the experiment; it was collected into about 0.5 c.c. of a 10 p.c. sodium-citrate solution and centrifuged. The plasma thus obtained was filtered in a micro-ultra-filter of de Ward(6), the urea in the ultra-filtrate was determined in both portions and in the latter the percentage of dye also estimated. Moreover, at different moments after injection, catheterised urine was collected and of this both the content of dye and of urea was determined.

The percentage of dye was with a very sufficient degree of exactness estimated in a simple way by means of a colorimeter, as recently described by me (7). In most cases it was necessary not to make the standard solution with pure water; so the indicator phenol red was diluted with a weak alkaline solution. If, as occurs with indigo-carmin and fluorescein, the colour in albuminous solutions was of different shade, a constant quantity of serum was added to the standard dilutions for determining the concentration in the plasma. Urea was estimated by the urease method with colorimetric measurement of the formed ammonia. The apparatus for this was taken so small that 0.5 c.c. of the ultra-filtrate and of a 10 times diluted urine answered the purpose.

In this manner could be estimated:

- (1) the concentration of the dye present in the blood plasma during a certain period;
- (2) the concentration at which such a dye concentration could pass from the blood plasma into a glomerulus filtrate, free from colloids;
- (3) the minimal inspissation of the glomerulus filtrate in the tubules, based on the concentration of dye in the urine.

The following dyes were examined: fluorescein-potassium, phenol red, indigo-carmin, lithium-carmin, trypan blue and trypan red, in order of their diffusibility. This means that the first three diffuse well, the last only very little. The diffusibility was estimated by the quantity of dye passing out of a watery solution through an ultra-filter.

For the sake of brevity I shall not give a full account of each dye-

stuff that was investigated, but as in the table below, place side by side the average concentration in the blood plasma and in the urine. Broadly speaking, it may be said that during the periods of investigation the concentration in these fluids oscillated but little; in both increase in concentration occurred rapidly and for hours after remained fairly constant.

The figures in the table are self-explanatory. Whereas the inspissation, as measured by the urea concentration, was in general not higher than 10-25, the proportion between the dye concentration in the colloid free ultra-filtrate of the blood plasma and the urine, determined at the same time, is disproportionately much higher. Even when the concentration is least (500 as in the experiment with fluorescein), we yet obtain incredibly high figures, higher even than the maxima, mentioned by Cushny. But in other cases the proportions become absolutely impossible, especially with phenol red, lithium-carmin and trypan blue.

Lithium-carmin and trypan blue, dissolved in plasma, do not pass an ultra-filter at all, nor does an aqueous solution of lithium-carmin, not even in $\frac{1}{1000}$ concentration. Trypan blue in aqueous solution at a concentration of $\frac{1}{1000}$ lets its violet component pass through, and only very little of the chief staining part. Inspissation out of an ultra-filtrate free from protein would have to be very great. Phenol red, though very diffusible, is almost entirely attached to the plasma colloids, so that an ultra-filtrate, free from protein, would have to be inspissated about 8000 times, in order to equal the concentration of the dye in the urine of the rabbit, examined at this moment. Now in this case only 7 c.c. of urine were excreted in one hour. This would mean 56 litres of glomerulus filtrate in one hour, secreted from the blood, the impossibility of which is evident, since on the ground of Cushny's figures, we must assume that in one hour's time at most 2-3 litres of blood, i.e. $\pm 1.5-2$ litres plasma, passes through the kidneys of the rabbit, a part of which, be it perhaps a rather large fraction, passes the glomerulus membrane for a moment. From this it follows that the hypothesis of the primary glomerulus product being equivalent to an ultra-filtrate, free from colloid, is not tenable as judged by secretion of dye-stuffs. The fact tends to suggest a return to Heidenhain's conception. On the other hand, all phenomena point to a secretion of dye-stuffs by the glomerulus and on this account, as well as on account of the existence of glomeruli with their characteristic blood supply, I am not inclined to give up the principle of Cushny's view. But this is only possible by rejecting the fundamental idea of the primary glomerulus filtrate being free from colloids and by assuming that the membrane does let through those substances. The assumption, though I do not

TABLE I.

Exp. No.	Injection	Diffusibility of inj. dye: content in ultra-filtrate from watery solution	Concentration of injected dye in	Concent. of urea in ultra-filtrate of blood plasma mgr. in 100 c.c.	Concent. of urea in urine mgr. in 100 c.c.	Proportion protein free glomerular filtrate: concent. dye urine	Proportion blood plasma (albuminous glomerular filtrate): concent. dye urine	Proportion blood plasma: concent. urea in urine	Reabsorbed part of urea per cent.
I	Fluorescein								
	potassium								
	100 mgr.	100 %	.004	.0004	.2	0.50	1:50	1:22	56
	75 "	—	.0025	.0002	.1	—	1:40	—	—
	500 "	—	.055	.0066	3.0	560	1:55	1:31	45
II	Phenol red	.70 %	.003	.00004	.32	350	1:100	1:12	88
III	Indigo-carmino	35 %	.006	.00066	.7	—	1:115	—	—
IV	250 mgr. Lithium-carmino	0 %	.003	0	.066	—	1:22	—	—
V	Trypan blue 200 mgr.	From watery solution is ultra-filtered violet diluted dye	.0625	0	During the first hours violet colour in high concentration	560	?	1:12.5	—
VI	Trypan red 50 mgr. intravenously	From watery solution is ultra-filtered yellowish diluted dye	.025	0	Yellowish colour in urine	400	—	1:16	—

admit it without hesitation, does not I think make the theory of renal function any more complicated. If we assume the glomerulus membrane to pass all bound dye-stuffs, a concentration of 50-120 would have to take place (see Table), an inspissation falling within the limits of possibility, though of course further experiments with more dyes might reveal improbable concentration values. But this inspissation is still much greater than that we should have to assume on the ground of the urea concentration, simultaneously determined. From this it follows that a large part of the urea contained in the glomerulus filtrate must be reabsorbed again by the tubules. If then, we wish to adhere to Cushny's principle, we shall have to modify this theory as follows:

(1) The glomerulus is permeated by a fluid which contains a considerable part of the plasma colloids besides all crystalloids.

(2) In its further course this primary, diluted plasma loses a great part of its constituents by reabsorption in the tubules. This reabsorbed fluid contains not only the constituents of Locke's fluid, but also a variable part of those substances, which according to Cushny leave the body completely, as for example, urea. Reabsorption of substances, not normally present in the blood plasma, is so slight, that they are excreted fully.

I may make a few remarks on this theory:

Jean Oliver⁽⁸⁾ holds that the presence of urea in the cells of the tubules as found by a microchemical method, is a proof of the secretion of this substance through the tubules. This fact is, however, also accounted for by assuming that this substance is partly reabsorbed from the glomerular filtrate. This reabsorption was recently found by Mayrs⁽⁹⁾ on the basis of a comparison of the concentrations of urea, SO_4 and creatinine in the blood plasma and in urine.

The absence of absorption, or the very slight absorption by the tubules of substances which normally are not found in the blood plasma is in conformity with what is known of the behaviour of other cells in the body. It is clear that the body cells can make very little use of the substances which are not normal constituents of the body fluids.

It is probable then that they absorb none or very little of such substances, so that the behaviour of the tubule cells is only a special case of the behaviour of the cells of the body generally. But according to Abderhalden⁽¹⁰⁾ the body cells can learn to use foreign substances by forming synthetic ferments. Thus we might expect that in certain conditions the tubule cells would learn to absorb some substances not normally present in the blood. The condition appears to be the continued

hypothesis is right, the thin line would represent the curve of growth of the remaining testicle after unilateral castration performed prepuberally. This curve is the normal one removed to a certain degree to the left, *i.e.* the ascending part of the curve falling in an earlier period and both curves approaching gradually to the same horizontal line. Another hypothetical curve would also be in accordance with our assumption, a curve the ascending part of which would be not only removed to the left but also steeper.

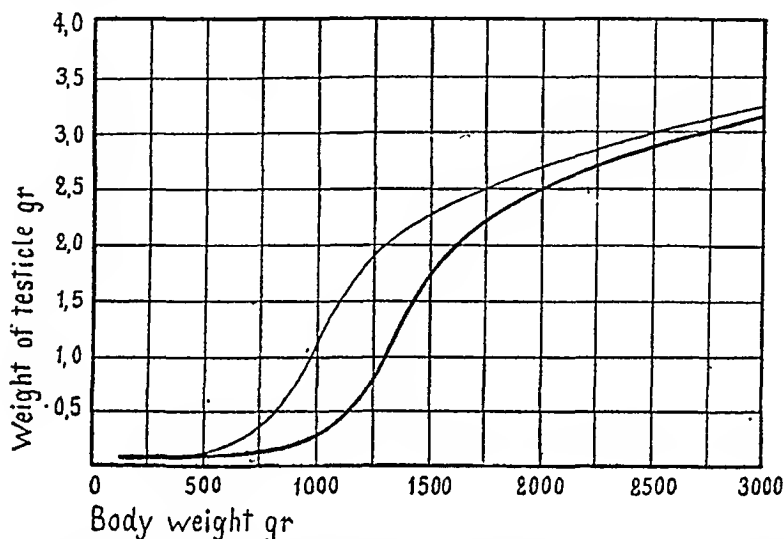


Fig. 1. Diagram to illustrate the hypothesis that the so-called compensatory hypertrophy of the remaining testicle is only an accelerated growth (see Text). The difference between a normal and a remaining testicle is supposed to increase gradually and after a maximal difference is attained at time of puberty to decrease again gradually.

The following experiments were made on these points: ten rabbits three to eight weeks old were subjected to semi-castration. The animals were etherized and the operation performed aseptically. In almost all cases the removed testicle was weighed without the epididymis and examined histologically. The preparations made by Dr Wagner showed the characteristic picture of the juvenile testicle. It may be said that according to our own numerous observations spermatogenesis begins in the rabbit in the third month; at the end of the third and at the beginning of the fourth month the seminiferous tubules enter into their rapid development to puberty. In the fourth month falls also the transformation of the juvenile penis into the adult form. There are naturally great variations as to the time at which these changes take place. Further, it seems very likely that in the rabbit, as in the guinea pig, puberty does

not mean stoppage of further development of the testicle and of the sex characters. There is also no close correspondence between body weight and weight of the testicle, as is seen from the diagram, Fig. 2. Out of the ten experiments performed we had in seven cases control animals of the same litter; in two out of the other three experiments the control animal unfortunately died.

TABLE I.

No	At operation			At end of experiment			Control		Difference between W and W' per cent.
	Body wt. grms.	Age in weeks	Weight of testicle, grms	Body wt. grms.	Age in months	Weight of testicle, grms. (W)	Body wt. grms	Wt of testicle, grms (W')	
I	190	3	.016	425*	3	0.023	420†	0.021	—
							450	0.030	—
							480	0.045	—
							610	0.016	—
II	280	3	.015	730	3	0.034	700	0.055	—
							720	0.076	—
III	140	3	.010	790	3	0.143	660	0.105	33.6
								0.108	—
IV	640	8	—	1250	4.5	2.65	1310	0.86 1.11	167.6 min. 138.8 max. 208.1
V	270	6	.019	1285	6.5	1.8	—	—	—
VI	290	0	.018	1310	6.5	1.43	1285	0.76	113.2
								0.75	min. 88.2 max. 140.0
VII	490	7	—	1880	14	2.57	1590	1.85	49.1
VIII	450	7	.030	1800	14	3.2	1730	2.45	min. 0.5
IX	480	7	.045	1880	14	3.84			max. 105.0
X	720	8	.076	1770	14	3.1	1750 to	1.1 to	—
							1800†	2.9	—

* Weighed several hours after death.

† Not belonging to the same litter as the experimental animal; the control animal died.

‡ Not belonging to the same litter as the experimental animal.

In Table I all data concerning these experiments are given. On comparing the weight of the remaining testicle of the animals I and II, in which the animals attained the age of about three months, with the normal testicular weight of animals of the same body weight, we see that there was no hypertrophy at all. The weight of the remaining testicle is within the limits of the normal variations. In Exp. III of the same duration the remaining testicle attained a weight greater than that in the control, the difference being about 34 p.c. The absolute difference

of 36 mgr. is very small and wholly insignificant if we take into consideration that the increase of weight in the testicle means development of the tubules and that some weeks afterwards there is normally an increase of weight of about 0.5 to 1 grm. In Exp. IV where the animals were kept for $4\frac{1}{2}$ months the remaining testicle attained a weight greater than both testicles of the control animals together or a weight 168 p.c. greater than that of the average testicular weight of the control. In Exps. V and VI the animals were kept for $6\frac{1}{2}$ months; the average difference between the weight of the remaining and the normal testicle was 113 p.c.

In Exps. VII-X the animals were kept till an age of 14 months. At this time it was found that in one control the scrotal sac was injured and the testicle adherent to the sac. In another control one of the testicles had remained in the canalis inguinalis. The animals were therefore killed at this stage. Histological examination showed that these two testicles were in a state of degeneration and their weights are therefore omitted from the table. It will be seen from the table that the average weight of the testicle of the operated animals in Exps. VII-X was 3.2 gm. and the average weight of the controls was 2.15 gm. a difference of 49 p.c. The same figure will be obtained on comparing the experimental animal X with the controls of the foregoing experiment.

If we go through the figures in the last column of Table I we see that the p.c. difference between the testicular weight of normal and semi-castrated animals is greatest at the time of puberty and becomes less in the following months. This result closely corresponds to our assumption graphically represented in Fig. 1, i.e. that after unilateral castration the hypertrophy of the remaining testicle is only an apparent one, its greater weight being caused by the removal of the curve of the growth to the left.

It might be objected to our results that in Exps. VII-IX the partial degeneration of the testicle on one side (the weights were 0.8 to 1.45 grm.), caused hypertrophy of the opposite testicle. In fact, however, the opposite testicles were much below the maximal of normal animals of like weight (cf. Fig. 2), and, as will be shown below, semi-castration does not in the adult cause hypertrophy of the opposite testicle. The following observation also indicates that there was no hypertrophy. The father of the animals in Exp. VII-IX was killed when $2\frac{1}{2}$ years old, its body weight being 2.5 kg. The testicles weighed 3.33 and 2.9 grm. and it will be seen from Table I that in only one of the three experimental animals was the weight greater than this.

It might also be objected that there are great variations in the testicular weight in different animals and in that of the same animal.

But if we compare the minimal and maximal differences of the different groups (Table I, last column) we see that there is a gradual decrease of the p.c. difference from group to group

It is of some interest that in the four last semi castrated animals the skin of the scrotal sack was extremely pigmented. This was evidently due to the increased metabolism of the testicles. Such an increase of metabolism may, however, be caused either by real hypertrophy or by accelerated growth

If the greater weight of the remaining testicle in castrated animals were a compensatory one, one would expect hypertrophy also when semi-castration is performed in adult animals. Nothnagel(6) performed such experiments on rabbits the body weight of which ranged between 1360 and 2470 gm. and Stieve(2) has taken them as showing compensatory hypertrophy. This interpretation is quite unjustified. In Nothnagel's experiments the maximal and minimal testicular weights after three and six months semi-castration were the same as before. It is true that the average weight was somewhat greater, but the absolute and percentage differences were insignificant, and in view of the fact that the normal testicle continues to grow after puberty, the small increase in average testicular weight in Nothnagel's experiments was in all probability simply the normal growth during the three to six months period.

Kyrle(7) made semi castration experiments on two fully grown dogs. The remaining testicles were removed three and six months after the operations and showed no macroscopical or microscopical differences from the normal testicles removed earlier.

TABLE II

No.	Age at operation in mths	Body wt. at operation grms.	Body wt. at end of exp. grms.	Age at end of exp. months	Wt. of testicle at operation grms.	Wt. of testicle at end of exp. grms.	Max testicle wt. in a normal animal of same body wt. grms.
I	3.5	950	1600*	6.5	—	2.49	2.6
II	3.5	1060	1570*	6.5	—	2.28	2.6
III	7.5	1405	2050	19.5	1.73	2.59	2.87

* Weighed seven days before

We have made some experiments on unilateral castration of rabbits near or after the time of puberty. The results are given in Table II. We see that in none of the three operated animals kept till an age of 6½ and even 19½ months did the testicle attain a weight greater than in any normal animal of the same weight (cf. also Fig. 2).

In experiments I made with Bormann(8) on rabbits removal of both testicles in the adult caused characteristic signs of castration, i.e. signs of loss of endocrine function. Since such signs do not appear

after semi-castration it is clear that the absence of hypertrophy in the remaining testicle in the adult after semi-castration is by no means connected with a lacking demand for sexual hormones.

Further evidence for our assumption is given by the following results. We weighed the testicles of normal rabbits of different body weight. As said above there are extraordinary individual variations. This is best seen in the diagram, Fig. 2. But notwithstanding all these variations it

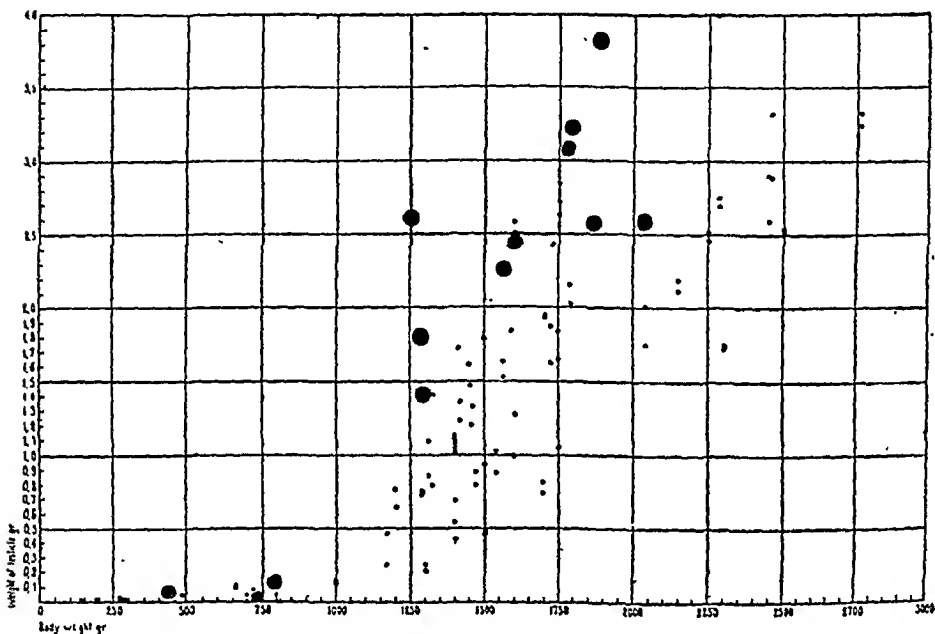


Fig. 2. Weight of 90 normal and 13 remaining testicles in rabbits (Tables I and II).
Small dots, normal; large dots, operated animals.

is clear that the curve of growth of the rabbit's testicle is rightly expressed in the thick line shown in Fig. 1. It may be noted that this curve is very similar to that given by Donaldson⁽⁹⁾ for the testicle of the albino rabbit; the main difference is merely that in the rabbit the ascending part of the curve begins later than in the albino rat. If now we compare with the normal testicular weights those of semi-castrated animals (clear circles) we find that the testicular weights of the latter are much greater than in normal animals at the time of puberty, whereas later on the weights are not very different. We see also that the weights of the testicles of the semi-castrated animals of a given body weight group do not in general surpass the maximal normal testicular weight of a higher body weight group. Out of 13 unilaterally castrated animals there was

only one (Exp. IX) where the testicular weight was greater than any of the normal animals observed. The same can be demonstrated for the guinea pig by using the figures of Halnan and Marshall's (10) experiments made for other purposes. In these experiments the testicular

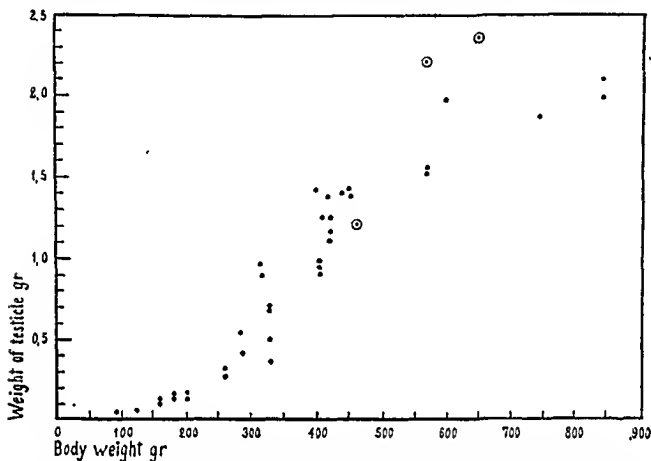


Fig. 3. Weight of 36 normal testicles in guinea pigs and of three testicles of animals from which the greater part of one testicle was removed. Dots, normal; circles, operated animals.

weights of semi-castrated animals surpass indeed the maximal normal testicular weights of the same group, but they surpass that of the following body weight group only in a very insignificant manner. The figures of Halnan and Marshall correspond to that part of our diagram, Fig. 2, which is between about 1000 and 1500 grm. One might object that in these experiments there was an operative interference of another kind and that the animals semi-castrated when of 117 grm. to 154 grm. of body weight were kept only for 51 days after operation. We have not made special experiments with semi-castration on guinea pigs but what we observed occasionally closely corresponds to what is shown by the figures of Halnan and Marshall. I removed, for other experimental purposes, the greater part of one testicle in three guinea pigs of 110, 160 and 240 grm.; the second testicle was left intact. As was to be expected the small testicular fragment left from the removed

testicle showed no hypertrophy although the experiments lasted more than four months. As these testicular fragments were extremely small the experiments are equivalent to experiments with semi-castration. The testicular weights of the experimental animals are compared with normal weights in the diagram, Fig. 3. We see here the same as in the diagram, Fig. 2, for rabbits between 1500 and 3000 grm. The testicular weights of two experimental animals are indeed surpassing the maximal normal weight; but the difference is not of any significance¹.

SUMMARY.

Experiments are recorded showing that the difference in weight as observed between the remaining testicle after semi-castration when performed at an early age, and that of a normal rabbit, is the smaller the older the animal and the nearer full testicular maturation.

Out of four unilaterally castrated animals which were under observation during more than a year only once the weight of the remaining testicle was greater than that of any normal testicles observed.

When semi-castration was performed on adult animals no hypertrophy occurred. No signs of deficiency of sexual hormones were present.

The facts clearly demonstrate that there is not only no compensatory hypertrophy of endocrine nature of the remaining testicle but that there is no hypertrophy at all. There is evidently another factor involved which causes an accelerated growth of the remaining testicle which in consequence attains sooner its maximal weight. The hypothetical curve as given in the diagram, Fig. 1, seems to be verified by the experiment although it was and will be impossible to verify each point of this curve on account of the extraordinary individual variations concerning testicular weight.

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¹ Since these experiments we have weighed the intact testicle of two other animals operated on in the same manner and kept up to the age of over 10 months. The body weight was 670 and 685 grms. The intact testicles weighed 1.8 and 2.0 grms.

ON GOLGI'S INTERNAL APPARATUS IN DIFFERENT PHYSIOLOGICAL CONDITIONS OF THE MAMMARY GLAND¹. By C. DA FANO.

(From the Physiological Laboratory of King's College, London.)

Soon after the publication of his arsenious acid and silver nitrate method, Golgi(11) gave a description of the apparatus of the mucous secreting cells of the gastric and intestinal epithelium of various vertebrates, but particularly of the frog's stomach. He showed that in the most deeply situated cells, in which the production of mucin is very active, the apparatus is relatively small and situated next to the pole of the nucleus facing the lumen of the gastric glands. In the cells, which are nearer to the surface and in which a great deal of mucin is accumulated, the apparatus acquires, little by little, a more robust appearance and is, at the same time, displaced beyond the nucleus towards the base of the cells. Though a fact of this sort may simply be due to the pressure of the mucin accumulating in the uppermost part of the cytoplasm, Golgi cautiously points out that there is no sufficient reason for stating or denying that the phenomenon is purely mechanical in character, or that it is or is not more intimately connected with the functional activity of the cells.

Golgi's observations were confirmed by D'Agata(9) who found that in the mucous secreting cells of the epithelium of the newt's stomach, the apparatus is, in normal conditions, situated between nucleus and free border of the cytoplasm, while in the cells surrounding minute experimental scarifications of the same epithelium, the apparatus is seen on the other side of the nucleus between this and the base of the cells. The author points out that this fact can be observed about 12 to 16 hours after the operation and that no mucin is present in the affected cells. He appears thus to suggest that the simple accumulation of secretion within the cytoplasm is insufficient to explain the shifting of the apparatus, and that some other biological activity may play a rôle in the production of the phenomenon. Some observations made later on by Kolster(13) and Basile(1) are in favour of this supposition. Kolster found that in

¹ The expenses of this research were in part defrayed by a Government Grant from the Royal Society.

the central cells of the deepest parts of the fundus glands of the rabbit's stomach the apparatus is small, very delicate in structure and situated very close to the nucleus, while in the cells of the more superficial portions of the same glands it is not so near to the nucleus and appears as a more robust network formed of rather thick and often varicose filaments. Basile saw that in the tubules of the remaining kidney after extirpation of the other, the apparatus has no longer the situation between nucleus and glandular lumen which was observed in the normal kidney by Brugnattelli(2) and is common to most epithelia even in pathological conditions. A similar fact was noticed by me(7) in a transplantable adeno-carcinoma of the rat, particularly in those parts of the tumour in which the acinous structure was best preserved.

These facts show that the morphological aspect and intracellular situation of the apparatus may be variously influenced by functional changes of the cytoplasm. A similar conclusion may be drawn from the many descriptions of the apparatus in various normal or pathological conditions, and during cell division.

From a physiological point of view, the observations of Cajal(3) on the goblet cells of the small intestine, and the investigations of Pensa(15,16) on the pancreas cells of *Triton cristatus* are by far the most important.

The goblet cells of the small intestine, says Cajal, soon after having discharged the elaborated mucin, are in a sort of atrophic condition characterised by their elongated shape and rather scanty protoplasm which no longer reaches the free surface of the intestinal epithelium. At this time the apparatus consists of one or two small rods or clumps situated close to the nucleus on the side turned towards the free epithelial surface. As mucigen is elaborated in their cytoplasm, they considerably gain in size while the apparatus becomes larger and more complicated in structure. When the excretion phase is approaching the apparatus breaks into fragments which presently cannot be distinguished from the granular secretion stained like the apparatus in silver nitrate specimens. Soon afterwards both the products of secretion and the fragments of the apparatus are thrown off from the cytoplasm and the apparatus is then rebuilt "through assimilation and division of its ultra-microscopic elements." Somewhat similar observations were made by Cajal in the submaxillary gland and pancreas of young animals either in normal conditions or after stimulating the glandular activity by means of pilocarpine. However, he points out that in these organs such a direct transformation of the apparatus into part of the secretory

product could not be seen. As to the significance attributed to these observations, he expresses himself as follows: "When a period of great functional activity approaches, particularly if this manifests itself as a process of chemical synthesis, the argentophil material forming the apparatus hypertrophies. In glandular epithelia hyperactivity is associated with a partial fragmentation and destruction of the apparatus."

Pensa found that in the pancreas cells of fasting newts the apparatus consisted of a net with small meshes and was situated between the nucleus and the apex of the cells. In the fed animals the apparatus was larger, had larger meshes and extended to some extent between the secretory granules. These changes were still greater after giving pilocarpine, the influence of which was already noticeable 3-10 minutes afterwards. In glands taken 30 minutes after giving pilocarpine the apparatus had extended further between the secretory granules and at later periods it broke up into minute reticular portions some of which were united by thin filaments. But Pensa does not mention any transformation of the apparatus into secretory granules. After the pilocarpine had ceased to act the network was even smaller than in the fasting animal.

The present research was planned with the object of finding out whether one or the other of the changes of the apparatus mentioned in the foregoing notes could be clearly observed in epithelial cells undergoing some well-defined functional modification.

Method. The mammary gland of small laboratory animals was chosen as one of the most suitable materials for the purpose, since the secretory activity of its cells is, in certain periods, very great and almost continuous. The bulk of the research was made on mammary glands of mice and rats, which are easily obtained at known periods of pregnancy and lactation. Material for comparison was collected from cats, rabbits and guinea-pigs, from animals either grown in laboratories or of which the age and functional condition had been, at least approximately, ascertained.

All mammary glands were fixed and treated according to my cobalt nitrate method (4, 5), taking care to reduce in general the time of fixation to about four to five hours, and in the case of glands from lactating animals to even shorter periods. The special precautions to be taken in the case of mammary glands in a state of involution after lactation, will be considered later on. All pieces were cut in series and the specimens toned and counterstained as described in previous papers (5, 6).

A search in the literature of the subject was made but no proper description of the apparatus in the cells of the mammary gland was

found. The only papers in which it is briefly mentioned are one by Tello⁽¹⁷⁾ and one by Kolmer⁽¹²⁾. The observations of Tello were made when investigating the internal apparatus of a benign adenoma of the mammary gland. This consisted, almost entirely, of glandular acini lined by racket-shaped epithelial cells, the widest part of which was turned towards the alveolar spaces and contained an apparatus comprising two or three finely reticular portions situated at the sides of the nucleus. A similar apparatus was found in the smaller cells arranged between the tapering ends of the racket-shaped cells and in those parts of the tumour which were structurally like the normal mammary gland. In some places the alveoli were lined with large cubical cells which also formed, here and there, simple agglomerations. The apparatus of these cells was hypertrophic and sometimes so large as to occupy a great part of the cytoplasm. According to Tello the hypertrophy of the apparatus was connected with the secretory activity of the cells and was followed by a phase of disintegration coinciding with a further accumulation of secretion within the cytoplasm. A similar fact, he says, was noticed in the glandular alveoli of a normal lactating mammary gland. Tello's results do not agree with those obtained by Kolmer, who succeeded in impregnating the apparatus within the cells of the mammary gland of a lactating guinea-pig. He found a distinctly reticular structure, the situation and aspect of which did not seem to have been affected by the droplets of fat accumulated within the cytoplasm.

The mammary gland of very young animals. In mice and rats about 10 or 12 weeks old, the apparatus of the epithelial cells lining the scantily developed acini is formed of one to three and sometimes even four small portions, situated close to the nuclear membrane, generally on that part which is turned towards the lumen of the alveoli. As shown by Fig. 1 each portion has a finely reticular structure and the portions are either independent one from the other, or, less frequently, united by thin threads. Within the glandular lumina some material similar to a coagulated serous secretion is frequently seen and, conglutinated with it, remains of altered cells and nuclei. Such appearances convey the impression that, as the gland develops and alveoli are formed, some of the cells lining their walls fall within the lumen where they undergo regressive changes and are finally reabsorbed. Much attention has been paid to these altered cells with the object of finding out whether the apparatus had disappeared previously to their mixing with the secretion; but this was not the case. On the contrary, as long as a nuclear membrane existed definite remains of the apparatus were present.

In still younger mice and rats, viz. of an age at which true mammary tissue has not been formed and only ducts in close proximity to the future nipple are found, the apparatus does not essentially differ from that described above except that the portions are smaller; they look, in general, like very minute and irregular rings or clumps of argentophil material. This fact is quite in agreement with the observations made by Marcora (11), Fañanás (10) and Cajal (3) in other kinds of developing tissues.

The apparatus of the mammary gland of very young guinea-pigs, rabbits and cats was found to be almost identical with that seen in mice and rats seven to ten weeks old. Similar results were obtained from the mammary gland of a 2-years-old rabbit which having, by chance, grown in isolated captivity, was certainly in a state of virginity. As shown by Fig. 2 the apparatus was only somewhat more developed than in the case of quite young animals of the same or different species, and was formed of small portions situated at the sides of the nucleus as observed by Tello (17) in parts of the benign human adenoma previously mentioned. However in this adult rabbit the whole gland had reached a high degree of development and its acini were in many places filled with a rather dense secretion containing a relatively great number of more or less altered detached epithelium cells (Fig. 2). In many of them the presence of typical remains of the apparatus was even more easily ascertained than in mammary glands of quite young animals.

Pregnant animals. When pregnancy supervenes the mammary gland increases in volume and its acinous structure becomes more apparent than in ordinary conditions. This is due to the rapid formation of a great number of acini, and is easily seen in mice and rats as early as the fourth or fifth day of pregnancy. At this period the apparatus looks larger than in mammary glands of young animals of the same kind. The phenomenon is quite plain a few days later when one can also remark that the apparatus is less frequently formed of separate portions. Fig. 3 shows this, as well as the unchanged situation of the apparatus next to the inner pole of the nucleus. In Fig. 3 one can likewise observe that at the tenth day of pregnancy in mice (and the same is true for rats) the cytoplasm of most cells is distended by large drops of fatty secretion which do not seem, however, to have any disturbing influence either on the intracellular situation or on the structural aspect of the apparatus.

At about the 15th day of gestation, which in mice and rats is an already advanced stage, the most striking feature is the hypertrophy of

alveoli, karyokinetic phases were more easily seen than in mammary glands of mice and rats. Three of such phases are reproduced in Fig. 8 showing that during mitotic cell division, in the mammary gland as in other tissues, the apparatus breaks up into minute particles which, after distribution between the daughter-cells, rebuild within each of them a new apparatus. The successive phases of splitting and rebuilding need not be fully described here, as they do not differ from what has been previously observed by other authors in various tissues and by myself in transplantable tumours of the mouse, rat and guinea-pig (7).

Within most alveoli of all the investigated mammary glands from pregnant animals a remarkable amount of fatty secretion is found, steadily increasing from the beginning to the end of pregnancy. Mixed with the secretion more or less altered cells are seen, just as in the case of non-pregnant animals. As previously pointed out, most of them look like detached epithelium cells and are provided with recognisable remains of the apparatus. In other places irregular and sometimes reticular fragments or clumps of argentophil material are often observed without any apparent connexion with altered cells. The aspect of these fragments is such as to suggest that they may be due to a further degree of disruption of the apparatus.

In consideration of the results obtained by Cajal (3) and Pensa (15, 16), many serial sections of mammary glands from animals at different periods of pregnancy were carefully examined with the object of finding out whether any evidence could be obtained as to a supposed transformation of the apparatus into a sort of granular material which would then be thrown off from the cells together with the other products of their activity. But no fact could be anywhere observed in favour of such an assumption. However, one feels justified in suggesting that the reticular clumps and fragments of argentophil material mentioned above represent a sort of indirect participation of the apparatus to the secretory function of the glandular epithelium, in so far as they can be considered as broken remains of the apparatus of cells fallen *in toto* into the alveolar lumina. Such a supposition is in agreement with the fact that within many alveoli not only recognisable epithelium cells but also badly stainable remnants of nuclei and particles of protoplasm are found. The observation that the minute reticular clumps of argentophil material are much more numerous in milk ducts at the end of pregnancy, and the most accepted view as to the origin of the colostrum corpuscles indirectly support the opinion here expressed.

Lactation. If animals, for instance mice or rats, are killed at the

moment in which the young are born but have not been suckled, sections from the mammary gland show a picture quite different from that found

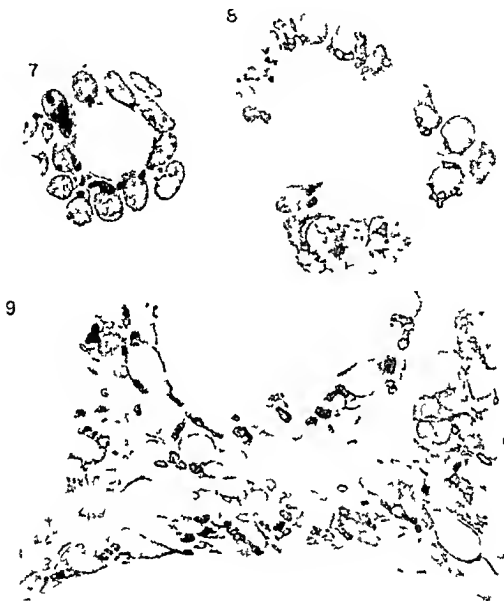


Fig 7 Guinea pig at about the 14th day of pregnancy Apparatus still small and normally situated

Fig 8 Guinea pig at about the end of the sixth week of pregnancy Moderate enlargement of the apparatus, broken up into minute particles in mitotically dividing cells From three different points of the same section

Fig 9 Rat killed at the moment the young were born Apparatus less noticeably hypertrophied than during pregnancy, shifted and stretched in various directions

in the last days of pregnancy As seen in Fig 9 the alveoli are dilated to an extent hardly reached even during lactation, the alveolar walls are lined with variously shaped cells the cytoplasm of which is almost

entirely occupied by milk globules. The apparatus is somewhat smaller than in the previous stage and completely shifted from its usual position next to the inner pole of the nucleus, to this or the other side of the cells. Its reticular structure is still maintained, but its shape is greatly modified, being sometimes that of a roundish body, sometimes that of an angular or irregular patch of argentophil material. More frequently it is elongated and stretched in the direction of the long axis of the nucleus. As pointed out in a preliminary note on the subject(8) these facts are very likely connected with the increased intracellular pressure due to the rapid production and accumulation of secretion within the cytoplasm. Fig. 9 is

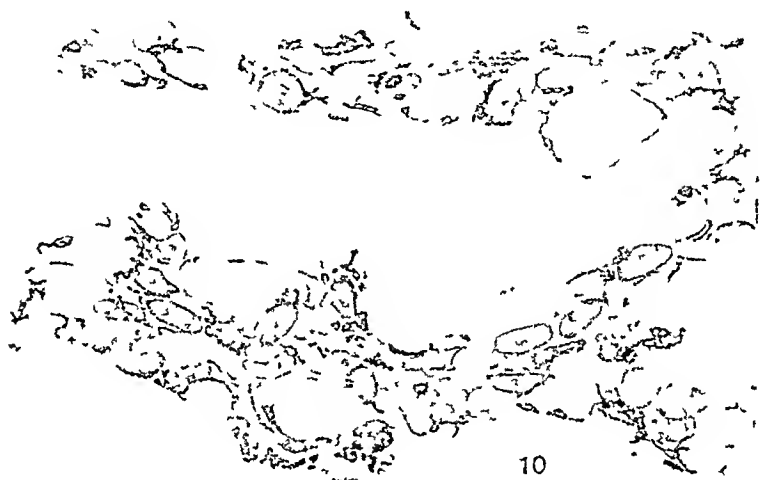


Fig. 10. Lactating rat. Maximum of shifting and stretching of the apparatus, which is more filamentous than reticular in character and partially fragmented.

only an instance of these various changes which, with some slight variations, formed the characteristic feature of all mammary glands investigated at this stage. A similar picture was exhibited by sections of mammary glands from lactating animals independently of the length of time from which lactation lasted. As shown by Fig. 10 the same facts as above described are observed, though in an exaggerated manner. The shifting and stretching of the apparatus, the irregularity of its situation and shape are certainly greater than in the previous stage. Even its structure seems frequently altered, being less distinctly reticular and more filamentous or even granular in character.

In serial sections from lactating mammary glands a search was again

made for some proof of a possibly direct participation of the apparatus in the secretory act, but nothing of the sort could be seen, neither a mixing of the apparatus with the secretion as described by Cajal, nor its total disappearance from cells at the height of their physiological activity as admitted by Tello. One must, however, recognise that in the lactating mammary gland the apparatus is, on the whole, smaller than in the same gland during pregnancy and frequently so fragmented as to justify the conclusion that in glandular epithelia hyperactivity is associated with a partial fragmentation and destruction of the apparatus.

The period of involution after lactation This part of my investigation presented at first some technical difficulties due to the necessity of altering the time of fixation in the cobalt nitrate and formalin mixture according to the state of involution reached by the gland and, to a certain extent, to the kind of animal from which the material was obtained. For instance, mammary glands from guinea pigs and cats are ripe for impregnation with silver nitrate after 6 or even 7 hours' fixation while in the case of mice, rats and particularly rabbits, 2 or 3 hours are sometimes sufficient. It is, therefore, advisable to collect many small pieces of the glands and to transfer some of them into the silver nitrate bath every hour starting from the first or second after fixation and continuing up to the sixth or seventh hour. It was also found that in the case of regressing mammary glands it is imperative that the mixture of cobalt nitrate and formalin should be prepared with neutralised formalin, *e.g.* shaken first with calcium carbonate, filtered and then re-shaken with animal charcoal. If these precautions are not observed it frequently happens that the apparatus does not stain at all, while the silver is taken by the glandular stroma. These variations in reaction may, perhaps, be connected with an altered reaction of the glandular tissue after lactation has ceased but the gland has not, as yet, returned to its normal state of rest.

If mice or rats are killed a few days after having been separated from their young no important changes are noticed in sections from the mammary gland. The alveoli are still greatly distended and filled with milk, and the apparatus has almost the same characteristics as described in the foregoing chapter.

Some days later, *viz.* towards the end of the first week after lactation has ceased, the following alterations are observed: the alveoli are still dilated though less than in the previous stage, some of them are empty, others contain a considerable amount of secretion which seems somewhat thicker than during pregnancy and lactation, the number of detached

great deal of intensely stainable cytoplasm as if some material, no longer wanted for physiological purposes, had accumulated within the cell body.

Similar facts are shown in Fig. 13 from a section of a guinea-pig's mammary at a more advanced stage of involution. The specimens of this series had a peculiarly light aspect possibly because the guinea-pig had been kept under the influence of ether for a rather long time and killed with it. But apart from this detail, to be discussed in another paper, the large cells provided with roundish or oval shapes (A of Fig. 13) were almost identical with those observed in the previous stage (cf. with Fig. 12). The shapes were, however, more clearly defined against the cytoplasmic network and the argentophil material from which they were delimited more distinctly filamentous in character. At this stage the glandular lumina were smaller than in the previous one and in places almost entirely filled with cells or fragments of cells of the type just described. This was even more obvious in microscopic fields comprising parts of still distended ducts, where rows and conglomerations of such cells could be seen. In B of Fig. 13 some cells are shown which were provided with an apparatus similar to that of the resting condition and beginning of pregnancy in guinea-pigs (cf. with Fig. 7). In the cells shown in C the apparatus had, instead, an intermediate aspect between that of the same structure in normal conditions and the peculiar shapes of the large cells.

This phase of the glandular regression lasts several days in guinea-pigs. With slight individual differences it begins between the fourth and sixth day and continues till about the end of the second week from the time in which lactation stopped. All through this phase pictures similar to that shown in Fig. 13 are very frequent. But, as the involution proceeds, the large cells rapidly diminish in number and finally disappear. The predominant feature of most specimens is therefore that shown in Fig. 14. The glandular lumina are much reduced in size and, as a rule, empty; all cells are generally smaller than in the previous stage; the apparatus has, in the main, reacquired its usual aspect (A of Fig. 14); only, now and then, cells are found provided with transitional forms similar to those above mentioned (B of Fig. 14; cf. with C of Fig. 13).

Can the so-called peculiar shapes be definitely considered as an apparatus? A decisive answer to this question cannot be given at present. As far as I know nothing of the sort has been previously described. Transitional forms between a typical apparatus and the roundish, oval or elongated shapes have been observed, but one cannot attribute to them a decisive value. They are, however, suggestive and a somewhat



Fig. 13. Guinea-pig, killed with ether, seven days after lactation ceased. A, altered cells with peculiar shapes as explained in Figs. 11 and 12. B, cells of normal aspect provided with an apparatus similar to that of the virginal condition and beginning of pregnancy. C, cells in an intermediate stage between those shown in A and those shown in B and provided with an apparatus of a transitional type between that of the normal cells and the peculiar shapes of the altered ones.

Fig. 14. Guinea-pig at the end of the second week after lactation ceased. A, cells provided with apparatus characteristic of the resting condition; B, cells with apparatus of transitional type similar to that shown in C of Fig. 13.



PROCEEDINGS
OF THE
PHYSIOLOGICAL SOCIETY,
December 17, 1921.

The acidity of muscle during maintained contraction. By H. E. ROAF.

In continuation of work commenced in 1912 the manganese dioxide electrode is being used to trace the relation of the hydrogen ion concentration to the tension of muscle. In contractions with normal frog's muscle the acidity disappears after the contraction but repeated stimuli seem to produce a greater and more continued acidity thus accounting for the greater tension in a tetanus.

In decerebrate rigidity the muscle is more acid than it is after it has been paralysed by cutting its nerve supply.

A simple method for the detection of nitrogen in physiological fluids. By H. E. ROAF.

By heating a liquid containing nitrogenous substances of animal origin with alkaline permanganate ammonia is given off. The ammonia can be detected by red litmus paper or by white fumes with the vapour of hydrochloric acid. Ammonium salts and to a lesser extent urea will liberate ammonia with alkali alone but alkaline permanganate will cause ammonia to be given off by many other substances such as amino-acids, uric acid, etc.

The proportions of the reagents may be varied but the following strengths are effective: 5 c.c. of the solution under investigation, 1 c.c. N NaOH and 1 c.c. 1 p.c. KMnO_4 .

A useful modification of the induction coil. By H. E. ROAF.

By means of a key the automatic interrupter can be short-circuited so that the coil can be used for single shocks or for repeated stimuli without altering the connections of the coil to the battery

The relation of methæmoglobin to oxyhæmoglobin. By H. E. ROAF and W. A. M. SMART. (*Preliminary communication.*)

Buckmaster has shown that methæmoglobin does not contain so much easily dissociated oxygen as does oxyhæmoglobin¹, nevertheless the total amount of oxygen in methæmoglobin may be the same as in oxyhæmoglobin.

To test this point methæmoglobin was made by the action of acetic acid on oxyhæmoglobin and the amount of gas given off was measured. The methæmoglobin so prepared seems indistinguishable from that formed by the action of ferricyanide. If the experiments are carried out so that the oxygen pressure is not reduced no gas is given off but if the pressure is reduced approximately half the volume of oxygen is obtained that is yielded by oxyhæmoglobin when acted on by ferricyanide.

The physical chemistry of these compounds and their relation to hæmoglobin is being investigated.

A note on the resonating system in the cochlea, with demonstration of a model, illustrating the action of a hitherto neglected factor. By GEORGE WILKINSON.

Assuming that the basilar fibres form a series of resonating elements, they will vibrate in accordance with the formula

$$n = \frac{1}{2l} \sqrt{\frac{t}{m}},$$

when n = number of vibrations per second,

l = length of fibre,

t = tension,

and m = mass of unit length.

We know that the basilar fibres are differentiated as to length, *i.e.* they increase uniformly in length from the base to the apex of the cochlea.

That they are also differentiated as to tension is extremely probable in view of the fact that they are attached to the outer bony wall of the cochlear galleries by a fibrous structure, the spiral ligament, which diminishes progressively in bulk and density from the base to the apex (Albert Gray).

¹ G. A. Buckmaster, *Journ. Physiol.*, vol. XLVIII, *proc.* p. xxv, 1914.

The writer's contention is that they are also differentiated progressively as to mass. In stringed instruments, *e.g.* the string board of a piano, the bass strings are increased in mass by being wrapped with copper wire. They are "loaded." Similarly the basilar fibres are loaded by a mass of cochlear fluid which moves as they move.

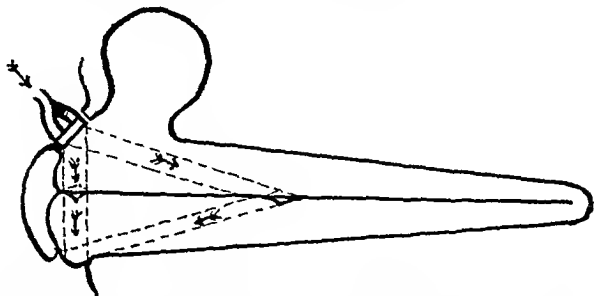


Diagram representing the cochlea unrolled. The two scalae are shown divided by the basilar membrane. Two sectors of the membrane are represented as vibrating. The fluid enclosed between the dotted lines represents the "load" on each sector.

Suppose a small transverse sector of the basilar membrane to move from its central position towards the scala tympani. Its movement will displace a certain amount of fluid, and the displacement will travel along the scala till the membrane closing the round window is bulged to a similar extent. A quantity of fluid equal to that displaced by the movement of the sector of the basilar membrane will be displaced across each cross section of the scala from the level of sector to the round window. A similar quantity of fluid will be displaced in the opposite direction in the scala vestibuli. The result will be the same whether the movement originates from an impulse applied to the stapes (forced vibration), or vibratory movements of the sector itself (free vibration). No sector can move without a simultaneous movement of the cochlear fluid. The mass of the fluid moved is quite definite and invariable for each sector. It does not depend on the amplitude of the movement. This only affects the amplitude of the displacement of fluid, not its mass.

The mass of fluid moved may be defined as that of a double column of fluid, the base of which is equal in area to the surface of the sector, and the length to the sum of the distances of the sector from the round and oval windows. The mass of the sector itself, with its adherent organs and cells, will be inconsiderable as compared to that of the fluid which

constitutes the load. Its specific mass will not differ greatly from that of the fluid in which it is immersed. The whole mass may therefore be reckoned in terms of that of the column of fluid. The fluid itself has a specific mass not greatly differing from water. In centimetre-gramme units the mass will be equal to the volume of the double column.

As m is mass of unit volume, it will not vary with the variations in transverse breadth of the basilar membrane. For sectors of the same width it will vary only with the distance of the sector from the round and oval windows. In other words m progressively increases from base to apex of the cochlea. This will cause a differentiation in the periodicity of vibration of the sectors in the same sense as the variations in length and tension. The load is greater on the sectors that are longest and least tense.

The formula for vibrating strings $n = \frac{1}{2l} \sqrt{\frac{t}{m}}$ when applied to the transverse sectors of the basilar membrane becomes

$$n = \frac{1}{2l} \sqrt{\frac{t}{db}},$$

where d is the sum of the distances of the sector from the round and oval windows, and b is the width of the sector.

The model shown is a brass box in two chambers (scala vestibuli with ductus cochlearis and scala tympani). A window closed by a rubber membrane opens into each. To one of the membranes is attached a small wooden plunger, the "stapes." The chambers are divided by a "basilar membrane" formed of strands of fine brass wire stretched transversely, and plastered over with fine paper saturated with formalised gelatine. The tension on the threads has been regulated by suspending from them a graduated series of weights, calculated according to the above formula. The whole is completely filled with water. It is set in resonant action by tuning forks applied to the stapes. Finely powdered blue enamel is used as an "indicator" to show the level of the vibrating segment. The compass of the strings is two octaves 100-200-400 D.V.

A new Drop-recorder. By N. E. CONDON.

This apparatus is represented in actual size in the figure. It consists of a spiral of thin platinum wire (No. 31) which is supported on a pivot with a small counterbalancing weight beyond it. The whole stands upon a brass rod carrying an electric contact screw. Between the spiral and the pivot is a projecting wire which dips into an isolated mercury cup

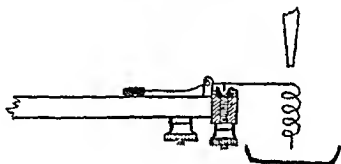
when the spiral is depressed, and thus closes an electrical circuit on which there is a signal. The spiral is fairly open and contains about four turns in its length—1 cm. It is protected by a curved brass plate which has been omitted in the figure. A drop of fluid falling from the pipette above depresses the spiral and completes the circuit. The drop runs through the spiral and is collected in a dish below for analysis.

The advantages of the recorder are:

(1) that it acts when the spiral is only 5–10 mm. from the point of discharge:

(2) that it records accurately the number of drops up to 180 per minute; and

(3) there is no loss of fluid by splashing.



PROCEEDINGS

OF THE

PHYSIOLOGICAL SOCIETY,

January 21, 1922.

Metal chamber for recording the rhythmic movements and tone of intestine. By B. A. McSWINEY.

The apparatus consists of a double walled chamber constructed of copper. The inner vessel serves to contain the tissue to be experimented on and has therefore its inner surface coated with tin, as this is found to avoid all injury to the tissues contained in it.

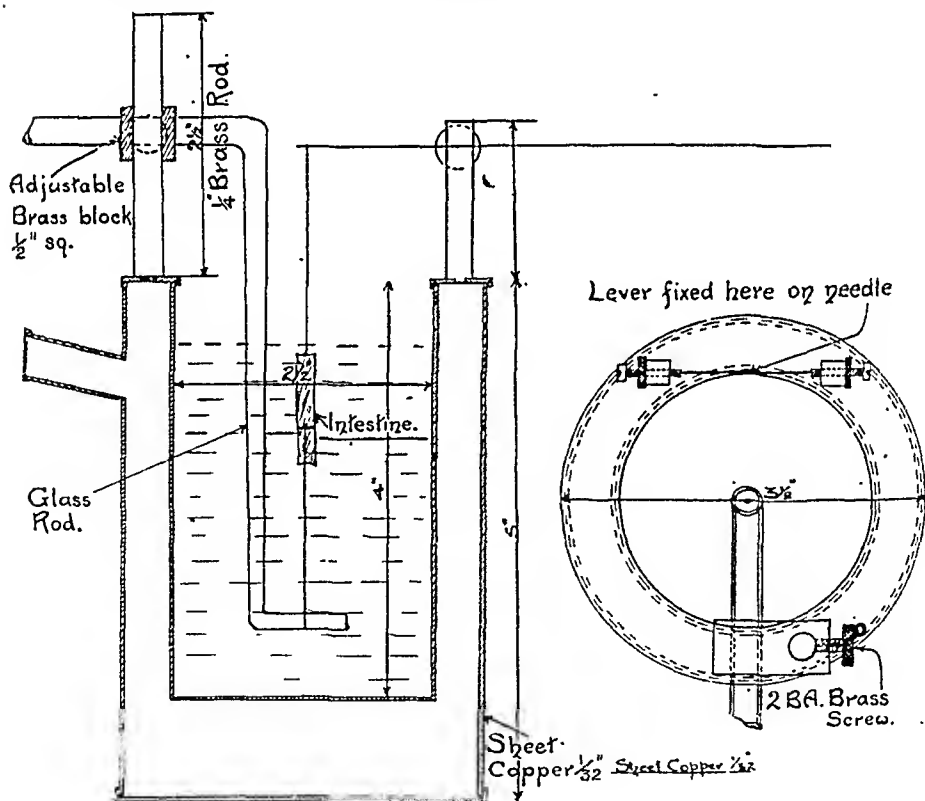


FIG. 1. Metal Chamber.

The outer vessel serves as a water jacket and is fitted with an inlet and an outlet tube so that the temperature may be controlled during the

experiment. Two metal uprights are fixed to the edge of the chamber and are fitted with a screw adjustment to hold a needle which forms the fulcrum of a straw lever. Opposite the uprights a clamp is fixed and holds a suitably bent glass tube which dips into the inner vessel. The piece of intestine or tissue is immersed in Ringer-Locke solution contained in the inner vessel, the lower part of the tissue being attached to the glass tube while the upper part is attached to the straw lever.

The apparatus has the advantage of being easily adjusted and being made of metal is unbreakable. The metal chambers are used by the students in the Experimental Physiology Laboratories, University of Manchester.

I am indebted to Mr A. C. Downing for the drawing.

The action of certain substances upon auricular fibrillation¹.

By T. LEWIS, A. M. WEDD and C. C. ILIESCU. (*Preliminary report.*)

If contacts are placed directly upon the chest wall over the region of the right auricle, the oscillations which characterise clinical fibrillation of the auricles may be recorded galvanometrically in a relatively pure form, and the rate of these oscillations can be counted within reasonable limits of error. The number of these oscillations per minute represents the number of circus movements which occur each minute in the auricle; these average 450 per minute.

This method of recording has been used to test or to compare the effects of certain substances upon fibrillation of the auricle in man.

Cinchona alkaloids. In testing and comparing these alkaloids, counts are made before and after the administration of single test doses under uniform conditions of rest, the counts being made at 15 or 20 minute intervals over periods of many hours. We find a conspicuous quantitative difference between the action of pure quinine and pure quinidine given by the mouth. While both these alkaloids lower the rate of the auricular movements, the action of pure quinidine is weight for weight much more powerful than is the action of pure quinine.

Commercial quinidine is usually contaminated by considerable percentages of hydroquinidine; a comparison between these two substances shows that their action is similar in character and not very dissimilar in degree. If there is a difference, hydroquinidine has, weight for weight, a slightly more powerful action than quinidine.

It appears to be a matter of indifference whether quinidine is given by the mouth in the form of base, or as sulphate, bisulphate or hydrochloride. The extent of the reaction and its time relations show no differences in these different circumstances. The action is independent of the solubility of the drug administered.

¹ Observations undertaken on behalf of the Medical Research Council.

Atropine sulphate, injected intravenously in doses up to $\frac{1}{10}$ grain produces usually a decided lowering in the number of oscillations, amounting to 30 or 50 beats per minute, exceptionally 100 beats per minute. This effect we ascribe to the removal of vagal tone, whereby the period of refractoriness is prolonged and conduction in the auricle tends to be slowed.

Digitalis. When cases of auricular fibrillation are brought fully under the influence of digitalis, there is usually a distinct increase in the number of oscillations per minute. Although this increase may not be seen in all patients, it frequently amounts to as much as 50 or 70 oscillations per minute. Digitalis bodies exert opposing actions; there is a direct action on the muscle, tending to prolong the refractory period and to impede conduction; and an indirect action through the vagus, which tends to reduce the refractory period and to speed up conduction; consequently, we are forced to ascribe the increase in the number of oscillations to predominance of the nerve action of the drug. In view of these observations, the well-known power of digitalis to convert clinical flutter into fibrillation, seems to be attributable to the action of the drug upon the vagus.

Respiratory Block 2 : 1. By H. ROBERTS. (*Preliminary communication.*)

By regulating the blood supply to the brain it is sometimes possible in the rabbit to halve or double the rate of breathing. In one case, apnoea having been produced by clamping both carotids and subclavians the right carotid was opened, whereupon respiration was resumed at practically half the original rate. In another case, the brain was fed by the right subclavian only. The right vertebral was then clamped, leaving only the collateral circulation. Respiration was halved. Again, adrenalin and pituitrin frequently cause the rate of respiration to be almost exactly doubled.

It is possible to show the gradual development of the doubled rhythm. For example, a rabbit whose brain was arterialised only by the collateral circulation (the two carotids and subclavians having been occluded) breathed slowly and regularly. On opening the left carotid these respiratory movements continued but alternating with them there appeared another set of movements at first shallow but steadily increasing until both sets were of equal depth. The rate of respiration was thus doubled. On clamping the left carotid respiration was suddenly halved. Whether the halving of the rate points to failure in initiation or in conduction of the nervous discharge is not yet determined. The facts seem to afford clear proof that the respiratory centre is automatic and not reflex and they suggest an interesting analogy with heart-block.

PROCEEDINGS
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PHYSIOLOGICAL SOCIETY,
February 18, 1922.

The effect of vagal stimulation on intra-auricular block produced by pressure or cooling. By T. LEWIS, A. N. DRURY, A. M. WEDD and C. C. ILIESCU.

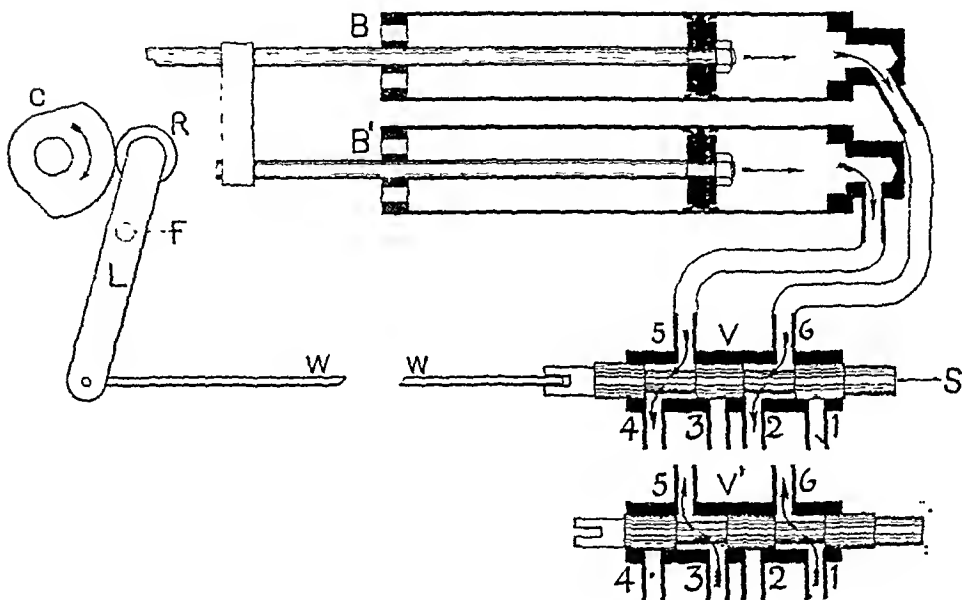
The auricular rate of dogs is controlled by rhythmic break shocks applied to the body of the auricle, and simultaneous records are taken from the body of the auricle and the tip of the right appendix. Regulated pressure is applied by means of a clamp across the base of the auricular appendix, thus producing various degrees of block between the body of the auricle and the right appendix; these blocks are recorded. The vagus nerve (right or left) is now stimulated and the ventricle ceases to beat (A-V block); at the same time the pressure block at the base of the appendix is *removed* in part or in whole. This effect is invariable, whatever the grade of pre-existing block (from a prolonged interval up to a complete block) provided that there is any change at all; in the last circumstance it is presumed that the pressure has also damaged the vagal apparatus in the clamped region. The reaction is not obtained after atropinisation of the auricle.

Similar effects of vagal stimulation upon intra-auricular block, produced in the same region by applying a metal tube containing flowing and cooled water, are obtained. Here again the block produced by cold is always relieved if any effect is witnessed; when there is no reaction it is presumed that the local vagal apparatus has also been damaged by cold. The same effects on the temperature-block are witnessed if, instead of stimulating the vagus, acetyl-choline is injected into the blood stream.

A simple double action respiration pump¹.

By E. H. J. SCHÜSTER, D.Sc.

The pump body consists of two barrels (*B* and *B'*) containing pistons each provided with two leather buckets facing in opposite directions. The piston rods are rigidly connected, and are given a reciprocating motion by means of a crank of adjustable length (not shown in figure), carried on a horizontal shaft. A cam (*C*) on the crankshaft



Diagrammatic section of pump (the individual parts are drawn to scale, but their arrangement is schematic).

actuates a sliding valve by means of the lever (*L*) and connecting wire (*W*). The valve and valve box are shown in horizontal section at *V* and *V'*. Nozzles 2 and 3 are connected with the trachea of the experimental animal. Nozzles 6 and 5 are connected with the barrels *B* and *B'* respectively. Suppose the pistons to be moving in the direction of the arrows, then the large diameter of the cam (*C*) holds the roller (*R*) towards the valve: the lower end of the lever is consequently held in the other direction and thereby the valve is maintained in the position shown at *V*. Then, by means of the two recesses turned in the valve, nozzle 6 is connected with nozzle 2, and 5 with 4. Air is therefore sent by barrel *B*

¹ Arrangements have been made with the Oxford Scientific Instrument Works, Wheat-sheaf Yard, Oxford, for the manufacture of this pump.

to the animal under experiment, while the contents of barrel *B'* are being exhausted. At the end of the stroke, the small diameter of the cam comes into operation, and thus by means of the pull of a spring at *S*, the valve is brought to the position shown at *V'*. It remains in this position during the suction stroke. Nozzle 1 is now connected with 6, and 3 with 5. In consequence, fresh air is sucked into barrel *B* and the respired air from the animal into barrel *B'*. It will be evident that any required gas-mixture can be administered by connecting nozzle 1 to a bag containing it, while products of respiration can be collected from 4.

Hæmorrhage as a form of asphyxia. By HOWARD W. HAGGARD
and YANDELL HENDERSON.

In a series of 53 dogs, blood was drawn at a standard rate to a low arterial pressure: 0.25 p.c. of body weight each five minutes down to 28–30 mm. mercury. This was done under cocaine without general anesthesia, and without pain, anxiety, or excitement. It was observed that many of the symptoms and processes which occur after hæmorrhage are identical with those seen in men and animals breathing under a progressively lowered oxygen tension or under progressive asphyxia with carbon monoxide. In particular the volume of air breathed per minute begins to increase after even a slight blood loss, and shows a progressive augmentation up to extreme air hunger. The minute volume of respiration is thus a convenient index of the severity of hæmorrhage. If the amount of breathing begins to decrease again soon after the hæmorrhage is ended, the subject recovers; if it increases, death results.

Corresponding changes occur in the blood. The increase of pulmonary ventilation, and reciprocal decrease of alveolar CO_2 , greatly reduce the CO_2 content of the arterial blood. A relative alkalosis (low ratio of H_2CO_3 : NaHCO_3) is thus induced. The organism then attempts to compensate for this condition by a diminution of the alkali in the blood, like that following over-ventilation and that recently described by us in men and animals under low oxygen and under carbon monoxide. Thus both the respiration and the blood CO_2 and alkali react after hæmorrhage much as they do during the development of acclimatisation to great altitude.

The restoration of blood volume by means of saline, equal in amount to blood drawn, is only temporarily beneficial; the statistics of recoveries were not much better than those of the untreated subjects. With gum

acacia solution (Bayliss) still better immediate effects, but no better ultimate results, were obtained. Sodium bicarbonate in 2 p.c. solution seemed to influence respiration, the blood, and the final outcome, somewhat more favourably. As re-infusion of whole blood would certainly have produced virtually complete recovery in practically all cases, the conclusion follows that it is the loss of red corpuscles which is the critical factor in hæmorrhage.

Insufficiency of red corpuscles seems to act through (1) inadequate oxygen transporting power, inducing hyperpnœa and the acapnial series of blood changes above described; (2) inadequate CO_2 transporting power, since it is the corpuscles which largely supply even the plasma with its capacity in this respect; and (3) inadequate capacity of the blood to produce NaHCO_3 from NaCl , and the consequent difficulty which the organism has in putting into effect the normal process for recalling alkali to the blood by decrease of breathing and relative acidosis.

These observations throw light on the similarity between traumatic shock without hæmorrhage and hæmorrhage without trauma in that both involve the acapnial process. The statement that "surgeons observed no excessive breathing in the wounded" is valueless, since even 100 p.c. increase of breathing is barely recognisable without measurement, and yet this increase involves a reciprocal reduction of CO_2 tension and blood alkali to 50 p.c. of normal, a condition scarcely compatible with life.

Permanent Golgi-Cox specimens. By C. DA FANO.

At the Annual Meeting of January, 1921, specimens were shown and a method was communicated regarding a simple way of rendering permanent Golgi-Cox preparations¹. The method consisted briefly of toning with gold chloride and fixing with sodium hyposulphite sections from pieces impregnated by the Golgi-Cox process and embedded in celloidin. Essentially one proceeded as in the case of Bielschowsky preparations and the like², the only difference consisting of a further treatment of the toned and fixed sections with alcohols of ascending strength to each of which one drop of saturated iodine tincture to every 5 c.cm. of alcohol had been added. This had been devised in order to remove traces of mercury chloride which might, in course of time, have given rise to an undesirable precipitation. The specimens were lastly mounted in xylol-balsam or colophonium under a cover-slip. It was shown that before

¹ Da Fano, *Proc. Physiol. Soc.* 54. p. cxiii. 1921.

² Da Fano, *Ib.* 53. p. xcii. 1920.

definite mounting the specimens could be counter-stained with alum carmine or some similar solution.

Some of the earlier specimens treated in this way are now exhibited again in order to show that, though mounted eighteen months ago, they have kept unaltered. In a few of them, however, the counter-staining with alum carmine has, little by little, faded away. I suppose that this is probably due to traces of iodine not completely removed by washing with alcohol. For this reason I have for many months abandoned the treatment of the toned and fixed sections with alcoholic solutions of iodine and adopted instead bleaching them with weak solutions of potassium permanganate and sulphurous acid as in Pal's modification of Weigert's myelin stain. One proceeds thus: Sections from celloidin blocks are collected in 70 p.c. alcohol, washed in distilled water and then toned and fixed as before with the usual slightly acidified 0.2 p.c. solution of gold chloride and 5 p.c. solution of sodium hyposulphite. After a thorough washing in distilled water they are kept for five or ten minutes in a 0.25 p.c. solution of potassium permanganate, washed in water, passed into a weak solution of sulphurous acid and washed once more in distilled water. There remain only counter-staining with diluted alum carmine, dehydrating and mounting under a cover-slip after clearing with carbol-xylol. This modification of the old process has many advantages: the bleaching does not affect the impregnation and renders the background much whiter and therefore more transparent than by the former iodine treatment; the counter-stain lasts longer, probably indefinitely; a great number of sections can be bleached, counter-stained and dehydrated at the same time, which is not without importance, particularly for class work.

On the microscopic appearance of human red blood corpuscles in hypertonic saline. By W. W. WALLER.

The apparent change is less with concentrated NaCl solutions (5 to 10 p.c.) than with weaker solutions (1 to 2 p.c.). The "crenation" effect is due to alkali dissolved off the glass under the microscope, when the glass surface is large compared with the volume of corpuscular suspension. Strong NaCl solution flattens the corpuscle with little or no change of diameter. Alkali causes a definite series of changes, observed and photographed. Its first effect is a change of the corpuscular shape towards the spherical. Its final effect is hæmolysis by a series of changes observed and photographed. In strong NaCl solutions the flattening

action predominates. In weak solutions the change towards the spherical by alkali predominates. The observations and photographs were made with the blood plasma practically at infinite dilution. A minimum hypothesis is suggested for these observations and deductions.

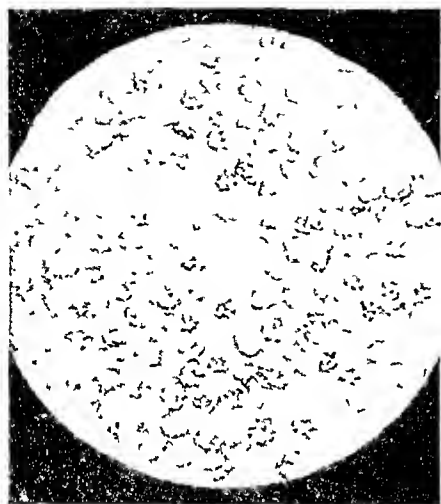


FIG. 1.

Photo. of corpuscles in 1.3 % NaCl, showing crenation (with decrease of diameter).

Magnification $\times 460$. $10\mu = \text{nearly } \frac{1}{2} \text{ cm}$



FIG. 2.

Photo. of corpuscles in 8.0 % NaCl, showing biconcave discs (with some curling of edges).

PROCEEDINGS
OF THE
PHYSIOLOGICAL SOCIETY,
March 18, 1922.

A valved trachea tube for respiration experiments.

By J. MELLANBY

The following valved trachea tube was designed for respiration experiments in which it was essential that the inspired and expired gases should not mix with one another.

A brass barrel *A* is fitted with a tube *E* which is inserted into the trachea of the experimental animal. The two tubes *C* and *D* are screwed into the two ends of the barrel, the tube *D* serving as a means of supplying any gas to the animal, and the tube *C* as an outlet tube for the expired air. When a record of the respiratory movements is required the tube *C* is attached to a tambour by a T piece, the third part of the T piece being closed to any degree required for a proper tracing by a piece of rubber tubing.

The valves placed in immediate proximity to the inlet and outlet tubes consist of a rimmed brass plate (*B*) perforated by six holes over which a thin piece of rubber membrane can be fixed by a central screw. When the rubber membrane is attached to the obverse or reverse side it acts accordingly as an inlet or outlet valve. The diagram shown is about the size required for cats.



Fig 1.

The type of respiration record obtained is shown by the two tracings, Figs. 1 and 3, which show respectively the effect on respiration of

breathing pure nitrogen for a brief interval of time, or a small quantity of carbon dioxide.

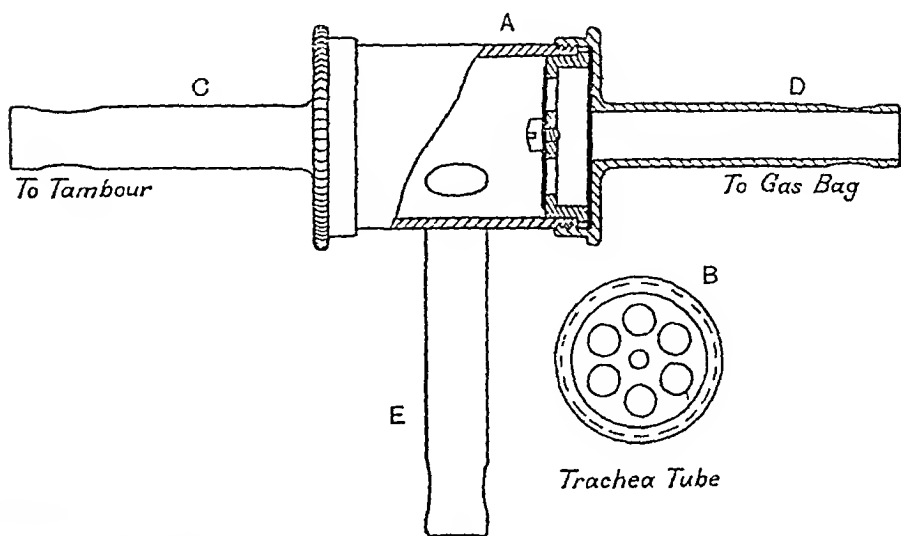


Fig. 2

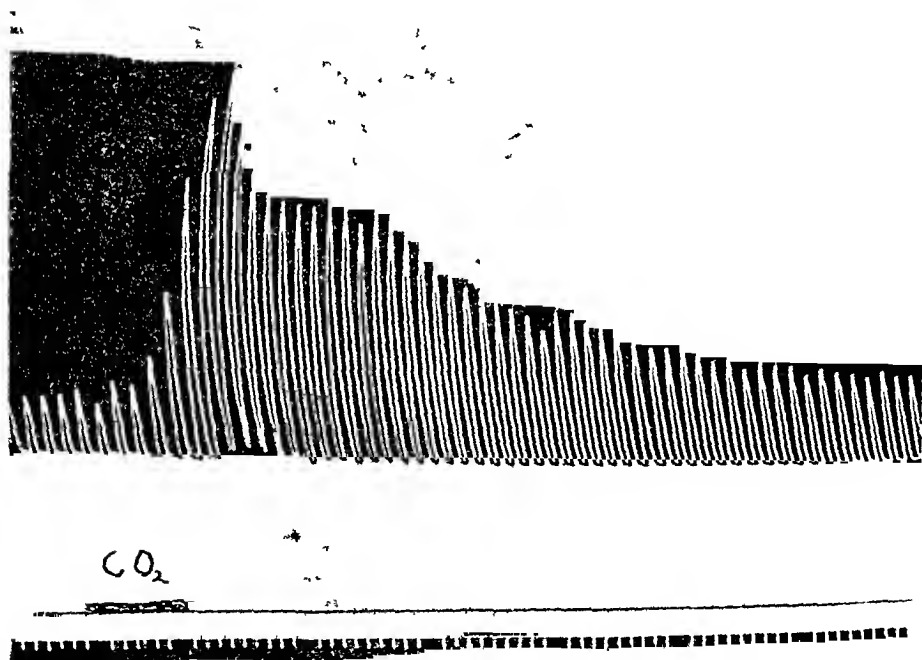


Fig. 3.

The recovery oxygen-usage after exercise in man. By H. LUPTON.

Experiments have been performed to determine the absolute extent of, and the time occupied in, the recovery from the high level of O_2 consumption, induced in man by muscular exercise. Three types of exercise have been considered:

(a) Prolonged moderate (walking up and down stairs for 20 minutes).

(b) Prolonged severe (running up and down stairs "all out" for 7-10 minutes).

(c) Short severe (jumping up and down as hard as possible, with a skipping movement, for 10 seconds, holding the breath).

All the types are followed, immediately on stoppage, by a rapid oxidative recovery. This has been followed by collecting the expired

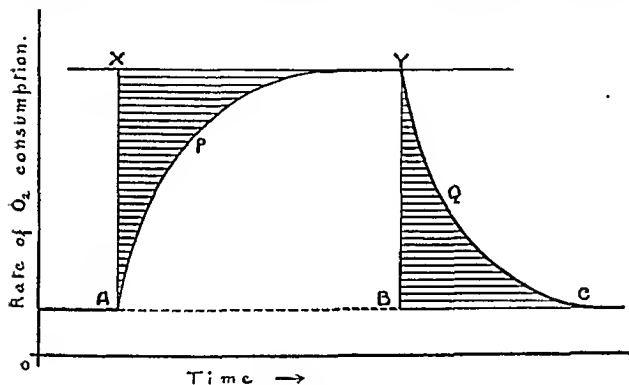


Fig. 1.

gases in Douglas bags during successive minute or half-minute periods, starting from the moment of stoppage of exercise. These various samples have been analysed by the Haldane gas analysis apparatus, and the O_2 consumed during the respective periods determined. The resting O_2 consumption preceding the exercise, and following the primary rapid recovery has been determined by the Sanborn Benedict respiration apparatus. In types (a) and (b) of exercise a Douglas bag was carried by the subject during the exercise, and a determination thus made of the O_2 consumption during the last minute of exercise.

The results indicate that in normal man the oxidative recovery from exercise is rapid and small.

An electrical method (katharometer) for measuring the CO_2 in respired gases. By A. V. HILL.

G. A. Shakespear (Birmingham) has devised an electrical arrangement for testing the purity of gases, depending upon the difference in cooling of a pair of balanced platinum spirals, by a standard gas and the experimental gas respectively. This instrument has been developed by the Cambridge and Paul Instrument Company, and in the form adopted for determining the CO_2 in flue gases serves admirably for the measurement of the percentage of CO_2 in alveolar air, or in other respired gases. The accuracy is high, and variations of temperature, humidity, and pressure are balanced automatically. Readings may be made rapidly and with practically no labour, and the instrument can be made in a portable set if required. It is possible to connect it with a recording galvanometer, so that a continuous record can be made of the CO_2 in a volume of gas, *e.g.* around a plant. Arrangements are being made to test it for clinical use at the Royal Infirmary, Manchester: and experiments are being made with it on respired gases, at the Physiological Laboratory. Its efficiency is largely due to the investigations of Dr H. A. Daynes, of the Cambridge and Paul Instrument Company.

The antagonism between histamine and adrenaline.

By C. H. KELLAWAY and S. J. COWELL.

Dale and Richards found that, in order to maintain the capillary tone of perfused tissues, the presence of traces of adrenaline in the perfusion fluid was necessary. They suggested that the normal function of adrenaline might be to maintain capillary tone by antagonising traces of depressant substances produced in metabolism or by cellular injury. Later, Dale showed that animals deprived of their suprarenals exhibited greatly increased susceptibility to the toxic effects of histamine.

In the course of some experiments on adrenal insufficiency, we have investigated the reaction of the non-anæsthetised cat to small doses of histamine. We find that the intravenous administration of $\cdot 1$ to $\cdot 05$ mgm. of base, or even smaller doses, causes not only the transitory slowing of the heart, dilatation of the pupils, sweating and salivation described by Dale and Laidlaw, but in addition a rise in the hæmoglobin percentage of the venous blood, almost as great as that observed by Dale in cats perfused with much larger doses of the drug. This increase in the hæmoglobin content is usually of the order of 10 p.c., and in normal cats passes off in from 20 to 30 minutes.

In cats dying of adrenal insufficiency a similar or greater concentration is demonstrable, but in these animals the effect is much more prolonged and usually persists for several hours.

In all the animals upon which these observations were made, one pupil had been denervated by the excision of the corresponding superior cervical ganglion, and responded preferentially to a dose of .001 mgm. of adrenaline chloride given intravenously. When .5 c.c. of a 1 in 1000 solution of this drug was injected subcutaneously into such an animal, the preferential pupil reaction appeared in a few minutes and remained evident as a rule for many hours. During this period small doses of histamine caused either no concentration, or only a trivial one. This antagonistic action of adrenaline occurs both in normal cats and in those with adrenal insufficiency. It should be stated that the adrenaline itself causes no alteration in the hæmoglobin value, either in normal animals or in those with high blood concentration resulting from adrenal deficiency. The general effects of histamine are not abolished by the previous subcutaneous injection of adrenalin. Animals with marked adrenal insufficiency are often prostrated by a minute dose of histamine: if adrenaline is first administered this does not occur.

We do not wish to claim that the prolonged concentration which follows histamine injections in animals suffering from adrenal insufficiency results from defect of medullary rather than of cortical function. We hope in a later communication to differentiate the part played respectively by loss of cortex and medulla in producing this effect, as well as the phenomenon of increase of the hæmoglobin percentage in the blood of animals dying of adrenal insufficiency.

A simplified air-analysis apparatus. By D. T. HARRIS.

The burette and absorption pipettes are made in one piece to do away with leaking rubber connections; this is firmly secured to a wooden stand. The potash and "pyro" reservoirs are easily detachable to facilitate cleaning.

The gas is manipulated by a single tap with borings and spot on the handle so arranged as to complete the whole analysis in one revolution in a counter-clockwise direction thus: with spot upwards introduce the gas, rotate 90° and measure sample, 45° to absorb CO₂, 45° to measure remaining gas, 45° to absorb O₂, and 45° to measure remaining N₂.

bodies, or more likely to a prolongation of the chemical processes of relaxation, the acid neutralised in relaxation gradually combining with the protein buffers of the intact muscle, a process which, according to Meyerhof, is accompanied by a considerable production of heat. Assuming "initial" (anærobic) heat = 1.0, anærobic delayed heat = 0.5, anærobic plus ærobic delayed heat = 1.5, the total anærobic heat becomes 1.5 and the oxidative heat 1.0. Accepting Meyerhof's latest estimate (370 calories) of the heat produced anærobically per gram of lactic acid liberated, we may calculate the following:

"Initial" anærobic processes	250	calories
Delayed anærobic processes	120	„
Delayed oxidative processes	250	„
Total	620	„

Meyerhof has shown recently that in the formation of lactic acid from glycogen, and in its neutralisation with phosphate or bicarbonate, there is a liberation of about 190 calories. This is almost large enough to account for all the "initial" anærobic heat-production. He has further shown that the neutralisation of 1 gram of lactic acid by buffered concentrated protein solutions (in which the ionised protein salt releases base to neutralise the lactic acid, itself forming the almost completely unionised protein acid) is accompanied by considerable heat-production, up to 140 calories. This heat Meyerhof regards as that of "unionisation" of the protein acid. If we suppose that the immediate neutralisation which occurs in relaxation (Ritchie) is due to phosphate or bicarbonate, and that in the absence of oxygen the muscle buffers slowly liberate base to absorb the acid so released and thus restore the alkaline salt, we have a quantitative explanation of the 120 calories produced in the delayed anærobic process. The same hypothesis will explain the fact that rest will restore, in the complete absence of oxygen, the quickness of relaxation of a muscle previously fatigued: the gradual restoration of the alkaline salts enables relaxation, which depends upon immediate neutralisation of the acid, to proceed more vigorously.

The total heat liberated in the complete cycle of production and oxidative removal of 1 gram of lactic acid, as calculated above, is 620 cals. This is only about 1/6 of the heat of oxidation of lactic acid. There is no doubt therefore that the lactic acid is not oxidised in recovery, but rather, as Meyerhof has shown, restored, to its precursor glycogen. The heat evolved is not sufficient to account for the oxidation of more than one molecule in six.

An automatic recorder of oxygen consumption¹.

By E. SCHUSTER.

This apparatus consists of (1) a water-sealed spirometer with a wedge-shaped hinged cap, (2) a pump which, when in action, delivers a known quantity of oxygen into the spirometer at each stroke, (3) mechanism for driving the pump. The trachea of the animal under experiment is connected with spirometer by two tubes provided with valves which ensure that the expired air passes into the spirometer through a container full of soda lime, and that the inspired air passes directly from the spirometer to the animal. The spirometer cap thus rises and falls as the animal breathes, and as the carbon dioxide is absorbed it falls a little lower at each breath until it reaches a point at which it closes a circuit containing an electro-magnet. The magnet when excited throws in a clutch which connects the driving mechanism with the pump. The pump gives one or more strokes before it is automatically thrown out of action, and thus raises the mean level of the spirometer cap. This process repeats itself with a frequency which depends on the amount of oxygen being consumed. Each stroke of the pump may be recorded electrically on a drum, and, as a writing point may be attached to the spirometer cap, a record of the excursions of the spirometer taken at the same time. The strokes of the pump are also registered on a counter attached to it, by which means the number delivered may be read off at any time.

The reaction of blood. By RUTH E. CONWAY and
FLORENCE V. STEPHEN.

A series of about 20 pairs of *p. H* determinations by the "dialysis method" on (i) whole blood, (ii) blood laked by freezing and thawing, and (iii) separated laked blood corpuscles, shows that at given equal carbon dioxide pressures, within the physiological range, the laked blood is more acid than whole blood by about 0.09*p. H*, and laked corpuscles more acid than whole blood by about 0.13*p. H*. The inside of the corpuscle therefore is about 35 p.c. more acid than the outside. This difference would be difficult to understand did not the presence of a semi-permeable membrane around the corpuscle, with some indiffusible ion inside and diffusible hydrogen ions on both sides, make possible the suggestion of A. V. Hill that a Donnan "membrane equilibrium" exists at the corpuscular boundary, and that an inequality of basic ion concentrations

¹ Arrangements have been made with the Oxford Scientific Instrument Works, Wheat-sheaf Yard, High Street, Oxford, for the construction of this apparatus.

produces an inverse inequality of the hydrogen ion concentrations. Comparative observations of the conductivity of (a) laked corpuscles, and (b) serum, have shown that, making all allowances for the effect of viscosity in lowering the conductivity of the former, the basic ion concentration is considerably greater in the serum than in the corpuscle, a fact which must necessarily lead to an inverse inequality in the *c.H.* The buffering of blood depends upon the amount of acid or CO_2 which can be taken up for a given rise in *plasma*-*c.H.*: it is improved therefore by the fact that the corpuscles are more acid than the plasma, and it has been found experimentally that the buffering of laked blood is less efficient than that of unlaked.

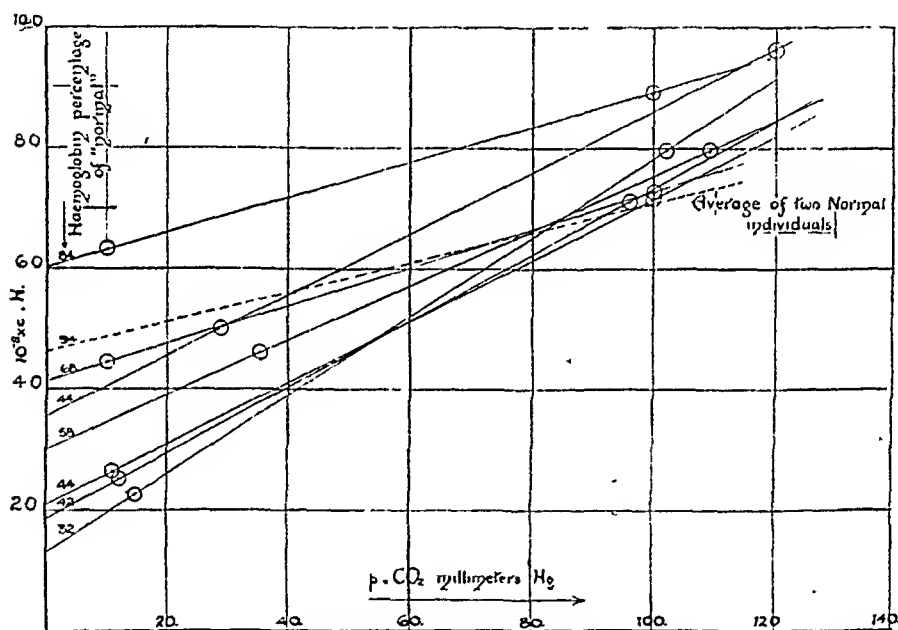


Fig. 1.

A study of the relation between the pressure of carbon dioxide $p.\text{CO}_2$ and *c.H.*, gives us in normal blood a line which does not pass through zero, implying that all base liberated at low CO_2 pressures is absorbed by hæmoglobin and phosphate buffers. When there is deficiency of hæmoglobin and phosphate to liberate base for combination with CO_2 or acid, or absence of corpuscular membranes to assist in absorbing excess of hydrogen ions, the *c.H.*- $p.\text{CO}_2$ line will be steeper than normal. The lines in Fig. 1 were determined directly on cases of pernicious or severe secondary anæmia, and they show an alteration in slope in direct relation

to the percentage of hæmoglobin present. Each line was drawn by determining directly two points upon it.

Further light is thrown on the subject by plotting the total volume of combined CO_2 ($v.\text{CO}_2$) against the observed $c.\text{H}$. This relation can be calculated from a knowledge of the $c.\text{H}-p.\text{CO}_2$ relations and the CO_2 dissociation curve, but has here been determined by direct $c.\text{H}$ estimations and by the use of the Haldane blood-gas apparatus for measuring $v.\text{CO}_2$.

Barcroft and his co-workers have found recently, in a study of normal individuals, that the $v.\text{CO}_2-c.\text{H}$ relation is linear over the range of physiological importance, and expressing it in the form

$$v.\text{CO}_2 = b.10^{-8}.c.\text{H} + c.$$

A. V. Hill has further shown that it is the constant b in this relation which measures the slope (and therewith the efficiency of buffering) while c is an expression only of the absolute amount of base uncombined with strong acids in the blood. Experiments are being made on the $v.\text{CO}_2-c.\text{H}$ relation in abnormal persons. It is clear that the efficiency of buffering cannot be improved simply by the addition of NaHCO_3 to blood, or clinically by injections of the same, and that to improve the corpuscular buffering power in the body transfusion of actual corpuscles is indicated.

A method of varying the frequency of stimulation.

By B. A. McSWINEY and S. L. MUCKLOW.

The method consists in the employment of an "eight cylinder" type magneto, belt driven from an electric motor. Both magneto and motor are fitted with pulleys of several sizes so that the speed of the magneto may be varied. Fig. 1 (p. xxviii) shows the wiring diagram of magneto and circuit.

When the armature of the magneto, which lies between the poles of a strong permanent magnet, is rotated, a current is generated in the primary winding. The primary circuit is broken, twice in every revolution of the armature, by the contact breaker (1). Each time the primary circuit is broken, a current is induced in the secondary winding. A "slip ring" and "pencil" carry the current from the secondary winding to a distributing board (2), in the magneto. The distributing board is fitted with eight metal segments, and for every four revolutions of the armature, one induction shock is distributed to each of the eight segments. By leading off from one, two, four or eight, of the segments, equally spaced

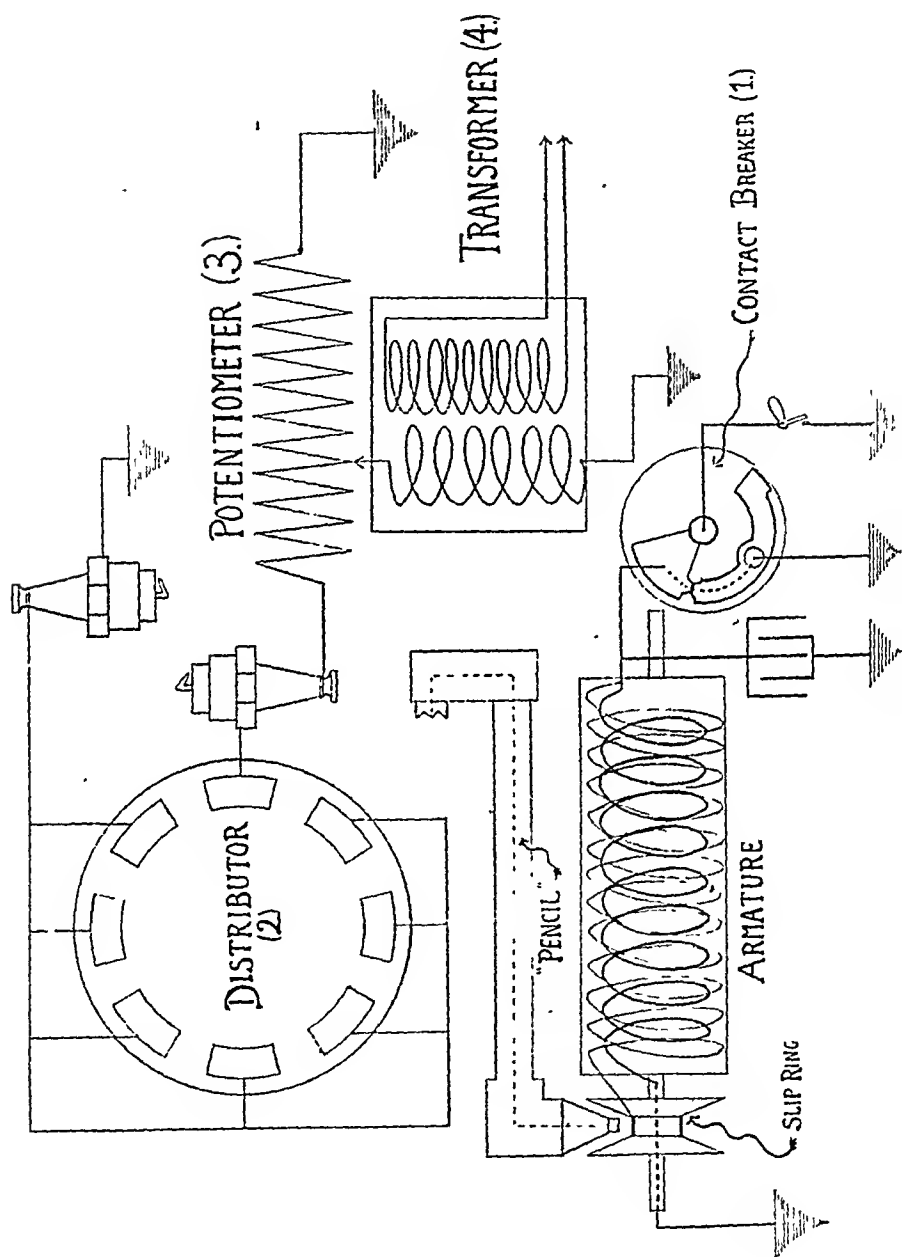


Fig. 1. Wiring diagram of magneto and circuit

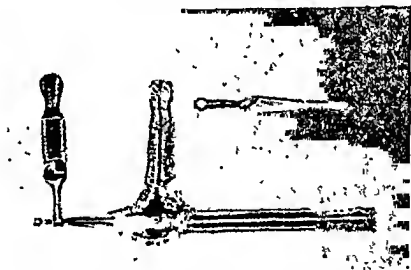
stimuli of four different frequencies are obtained for one speed of rotation of the magneto.

The current from the distributor is passed through a potentiometer (3) from which the required amount of current may be tapped off. A spark gap (consisting of an ordinary sparking plug) is interposed between the magneto and the potentiometer—this obviates the possibility of a small stimulus being obtained at "make" of the contact breaker, and also causes the "break" stimulus to be of a very short duration.

The unused segments of the distributor are "earthed" to prevent any possible damage to the insulation of the secondary winding, a spark gap is placed in the circuit, as this affords a convenient means of observing whether the apparatus is working correctly.

As the current obtained from the magneto is at a very high tension, it is passed through a transformer (4) which reduces the pressure in the ratio of ten to one. For the convenience of the operator a "short-circuit" key is placed in the primary circuit—when the key is closed the apparatus may be handled without any danger of the operator receiving an electric shock.

A clip-forceps for fixing cannulæ. By R. K. S. LIM.



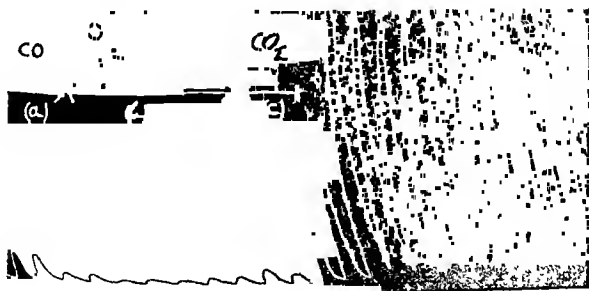
The accompanying photograph shows two clip-forceps, one viewed from the surface (forceps holding cannula) and the other from the side; the instruments are represented in actual size. The essential feature of the forceps is that the "biting" end is grooved; a selection of three such forceps with varying size of groove suffices for general use with the usual type of cannulæ. They can be employed for holding cannulæ or any small size tube in position in arteries, veins, ducts, etc. It is found that their

use instead of ligatures, *e.g.* with blood-pressure cannulæ, save time, causes less damage to the vessel and therefore minimises clotting. When clotting does occur, the clip can be released and the cannula quickly withdrawn to permit the ejection of the clot by the blood-pressure itself, after which the cannula is readily replaced. Lastly, the forceps does not slip and the portion above the groove may be used for temporarily clamping a vessel.

PROCEEDINGS
OF THE
PHYSIOLOGICAL SOCIETY,
May 20, 1922.

**The effect of carbon dioxide on respiration after poisoning
by carbon monoxide. By J. MELLANBY.**

After the respiratory movements of an animal have been diminished or annulled by carbon monoxide a marked stimulation of the respiratory centre can be produced by carbon dioxide. This stimulating action of carbon dioxide is in contrast to the absence of effect produced by the administration of oxygen. The tracing of the respiratory movements in an anæsthetised cat shows (a) the terminal stages of CO poisoning, (b) the absence of effect of pure O_2 , and (c) the marked hyperpnœa produced by the transient administration of CO_2 .



If the animal has ceased to breathe but the heart is still beating, deep respirations can be initiated by blowing a little CO_2 into the lungs. The administration of oxygen during the hyperpnœa produced by the carbon dioxide results in the rapid elimination of carbon monoxide from the animal's body, and the complete recovery of the respiratory mechanism.

The oxygen consumption during running.

By A. V. HILL and H. LUPTON.

The following observations were made by the Douglas bag method, on a subject running round an open-air track, 92½ yds. in circumference. In each of the experiments in Table I there was a sufficient fore-period (3 to 4 mins.) of running at the same speed, carrying the bag and breathing through the valves, to ensure that the rate of oxygen consumption had reached its full value.

The values of the oxygen consumption and CO₂ elimination are the highest recorded in physiological literature: the subject is of athletic build, 11½ stone (73 kilos), fairly fit, 35 years of age, and used to running: he is not however, and never has been, a first-class runner, and there can be no doubt that a champion "middle-distance" runner in training could attain considerably higher values (*e.g.* 5000 c.c. or more), especially if not subjected to the inconvenience of valves and bag. Such values are of great interest, showing a very high circulation rate and output of the heart during running: in the ease with which the circulation is maintained, running is superior to other forms of violent physical exercise.

The values of the oxygen consumption at 6.4 to 7.6 m.p.h. are "steady" ones: the subject could have continued running at such speeds indefinitely: the values at 9.1 and 10 m.p.h. are not "steady," the subject was using more energy than was accounted for by the O₂-supply, and was going "into debt" for oxygen. By measuring the total oxygen used in standing for seven minutes following five minutes running at 7.6 m.p.h., and subtracting the oxygen used in standing for a similar seven minutes after rest, we have found that the total steady "debt" of oxygen attained in such steady exercise is about 2500 c.c.: higher speeds cause considerably greater "debts," and very high speeds result in rapidly increasing (instead of steady) "debts," so that a stage is soon reached at which the accumulation of unoxidised lactic acid prevents further effort. The most severe exercise of which our subject is capable (analogous in violence to a 100 yds. race) led in 10 secs. to a total oxygen deficit of about 2500 c.c. and in 20 secs. to about 5500 c.c.: naturally such exercise cannot be continued for long: it represents an expenditure about 70 times his basal value.

In order to determine the rate at which the oxygen usage rises to its steady value during running, we have made experiments in which the subject collected various half-minute samples of expired air, after starting to run at a steady speed.

We see that the O₂- and CO₂-rates rise exponentially from the start, reaching a steady value within two minutes, the total deficit at the beginning of exercise being compensated in the early stages of recovery.

TABLE I.

Speed, miles per hour	6.4	7.4	9.1	S	10.0	7.6	S	7.6	S
O ₂ per mm c.c.	422	3490	4175	373	4055	3205	372	306	315
(O ₂ per mm c.c.)	356	3340	4475	328	4435	2905	301	269	275
(CO ₂ /O ₂)	87	96	1.07	88	1.07	91	82	88	87
Total ventilation litres per min	10.29	65.0	90.50	9.73	114.0	69.60	9.25	8.40	8.50

TABLE II

Speed in p.h.	7.6									
	S	7.0	1.9	2.47	3.62	S	15	31	56	80
Middle pt of sample, sec	373	2792	3300	3320	3205	306	1406	1933	2748	2846
O ₂ per mm c.c.	373	3340	3950	3950	4051	306	1406	1933	2748	2846
(O ₂ per mm c.c.)	328	3310	4040	4040	4335	269	1300	1616	2095	2370
(CO ₂ /O ₂)	88	81	91	90	91	88	92	84	82	83
Total ventilation litres per min	9.73	9.25	51.1	63.2	67.5	69.6	8.4	30.92	37.4	43.5
										47.2
										51.8
										55.9
										57.3
										8.5

S=Standing.

The influence of oxygen and carbon dioxide on the coagulability of blood. By J. MELLANBY and H. M. G. LESTER.

The capacity of peptone blood to carry CO_2 is much less than that of normal blood. Also the blood of a dog treated with peptone regains its coagulability within a few hours if left in the animal and *pari passu* with this return of coagulability the CO_2 carrying power of the blood returns to its normal value. The following figures illustrate this statement:

Plasma	Interval after peptone injection	% CO_2	Coagulation time
Original	—	63.5	2 mins. for original blood
Peptone I	At once	44	No coagulation
„ II	20 mins.	47	„
„ III	40 „	60	40 mins.

Experiments were made to determine whether the diminished CO_2 content of the blood was the cause or the result of the diminished coagulability.

Oxalated blood treated with an optimum quantity of calcium chloride has practically a constant coagulation time whatever its gas content, thus:

Oxalated blood	Coagulation time with optimum CaCl_2
Normal	1.5 mins.
Free from O_2 and CO_2	3.0 „
Saturated with O_2 ; free from CO_2	2.0 „
„ „ CO_2 ; „ „ O_2	2.5 „

The respiratory centre of an anæsthetised cat was put out of action by the injection of starch into the peripheral end of the left carotid artery. The following figures show the coagulation times, reactions and CO_2 contents of the blood before and after excessive lung ventilation:

	Coagulation time	p(H)	CO_2
Normal	3.25 mins.	7.32	38 %
Excessive ventilation (10 mins.)	5.5 „	7.31	14 %

A third series of experiments in which an anæsthetised cat was given N_2 or CO_2 to breathe gave the following results:

	p(H)	Coagulation time
Normal	7.31	1.25 mins.
N_2	7.37	0.25 „
CO_2	7.28	1.0 „

It is evident from the above results that the gases of the blood effect its coagulability to a small degree only. Large changes in the CO_2 content of blood produce alterations in coagulability in no way comparable to the effects observed in peptone blood. Apparently the particular change which diminishes the coagulability of peptone blood also diminishes its capacity to carry CO_2 .

The influence of bile salts on the rate of diffusion between two liquid interfaces. By B. J. COLLINGWOOD and V. G. WALSH.

If a burette be half filled with $n/10$ NaOH and $n/10$ acid be run carefully onto the surface of the alkali by means of a fine pipette, a junction line is established between the two liquids, which line can be rendered visible by the previous addition of a suitable indicator to the solutions.

This junction line exhibits when magnified an undulating surface resembling a mountain plateau. The peaks of this plateau may sometimes be seen breaking away and passing into the supernatant acid—a phenomenon which has been described by the term "intertraction" (Almroth Wright).

The line remains distinct for several weeks; but it is constantly changing in position. If H_2SO_4 , HNO_3 or HCl be the acid employed, the line for the first few days descends. If, on the other hand, CH_3COOH be used, the line rises.

The addition of $\frac{1}{8}\%$ of sodium taurocholate to the acid and alkali, although greatly lowering the surface tensions of the liquids as shown by the "drop" method, appeared to have no influence on the rate of movement of the line.

The descent or ascent of the line cannot be determined solely by the degree of ionization of the acids employed, for after two days it was found that the line between HCl and $NaOH$ had descended less than that between H_2SO_4 and $NaOH$, although $n/10$ HCl is more dissociated than $n/10$ H_2SO_4 . Again when a constant current was passed from the acid to the alkali it was found that the line descended more rapidly in the case of H_2SO_4 than of HNO_3 , although the dissociation of H_2SO_4 in $n/10$ solution is less than that of HNO_3 of the same normality.

In addition it was found that $n/16$ H_2SO_4 and $n/16$ HCl were in equilibrium with $n/10$ $NaOH$ in that the line remained stationary. This result again cannot be explained on a dissociation basis.

The amount of interchange between the acid and alkali was estimated in a number of experiments in some of which bile salts were present in the solutions. Here again no evidence was forthcoming that bile salts had any influence on the rate of this interchange. In short, we obtained no evidence that surface tension phenomena were involved in the process.

The alkalinity of the ultrafiltrate of the blood plasma.

By T. H. MILROY.

A method, other than those in common use, has been employed in order to supplement our knowledge of the potential alkali reserves of the blood. The method consists in the separation of the ultrafiltrate of blood plasma, and the subsequent estimation of the alkalinity of this colloid-free solution. Plasma ultrafiltrates of the following have been examined: arterial and venous blood, blood plasma evacuated, blood plasma through which carbonic acid has been passed, whole blood evacuated and whole blood exposed to the action of carbonic acid. In addition certain ultrafiltrates of blood plasma, which has been dialysed in various ways and then made up to different bicarbonate concentrations, have been examined with a view to determine the loss of alkali produced by dialysis.

The method of ultrafiltration enables one to separate the colloids with their store of alkali, be this large or small, from the readily diffusible bicarbonate and in doing so gives information regarding the part played by the colloids in maintaining the constancy of the blood reaction.

Briefly the procedure adopted was as follows. Horse blood was taken in order that sufficiently large quantities of the ultrafiltrate might be obtained for examination. It was taken directly from artery or vein into a vessel containing an accurately weighed amount of neutral oxalate, sufficient to raise the concentration in the blood to .3 %. The blood was run in under a layer of pure liquid paraffin. The subsequent treatment varied. In some cases the plasma was separated at once by centrifuge, and filtered through collodion plates at pressures rising up to $2\frac{1}{2}$ atmospheres. The first 80 c.c. ultrafiltrate were discarded but the later portion, 50 or more c.c., was kept for examination. Throughout the entire process the plasma was kept under the oil. The alkali value of the ultrafiltrate was determined in two ways: first, by titration against .02N H_2SO_4 using dimethylaminoazobenzene as the indicator for the neutralisation point of the bicarbonate (transition point p_{H} 4.2), and second, by conductivity estimations carried out during the process of neutralisation. Details of the latter method will be given in a subsequent paper. The conductivity method is suitable for the graphic representation of results, and also gives information regarding the nature of the substances acting as bases. The principle of the method depends upon the greater velocity of the hydron and, to a lesser extent, the

hydroxidion than the cation and anion of the neutral salt, so that on the first addition of acid to the ultrafiltrate there is a fall in conductivity owing to the decrease in the hydroxidion and subsequent to neutralisation a rapid rise due to the increase in the hydriion.

The neutralisation curves of pure bicarbonate solutions with or without the addition of NaCl show a rapid rise in conductivity at the titration end point, the increase occurring with the first pink tint in the fluid. In the case of plasma ultrafiltrates the rise does not occur until a later point, corresponding to a $\cdot 01$ higher molar concentration. That is to say at a reaction corresponding to p_H 4.2 substances other than bicarbonate act as bases, the reaction being on the acid side of their isoelectric point.

When the ultrafiltrates of carefully preserved arterial and venous blood plasma are examined in this way, as a rule a slight but distinct difference in alkalinity is to be observed, the venous plasma ultrafiltrate having the higher alkalinity. The difference is always less than $\cdot 005$ molar probably about $\cdot 002$. When separated blood plasma is charged with carbonic acid, the ultrafiltrate shows a rise in the alkali value to the extent of $\cdot 005$ to $\cdot 01$ molar and on evacuation of the plasma the value again falls to a lower level than that of the original plasma.

The average alkali values of the plasma ultrafiltrate of horse blood vary from $\cdot 03$ to $\cdot 04$ molar. It is quite evident that even in the case of the plasma colloids alkali may be withdrawn by carbonic acid and the reaction is a reversible one. The greatest variations in the alkali values of blood ultrafiltrates are to be observed in the comparison between evacuated whole blood and whole blood charged with carbonic acid. In this case the average values lie between $\cdot 02$ molar for evacuated and $\cdot 05$ to $\cdot 06$ molar for carbonic acid blood. The colloidal alkali reserve of the blood cells forms therefore a more important source of disposable alkali than that associated with the plasma colloids.

If before ultrafiltration the blood plasma be dialysed against $\cdot 85\%$ NaCl or against water and then sodium bicarbonate added to the dialysed plasma in an amount sufficient to raise the bicarbonate concentration to $\cdot 03$ or $\cdot 04$ molar, the ultrafiltrate subsequently obtained shows on neutralisation the same type of conductivity curve as the pure bicarbonate solutions. The alkali concentration is however less than that corresponding to the amount of bicarbonate added. This fixation of alkali by the dialysed plasma is most marked after dialysis against water.

The absence of relation between the amplitude of respiratory movement and the reaction of the blood. By J. MELLANBY.

In experiments on anæsthetised animals there is no obvious relation between the respiratory movements and the reaction of the blood, under varying experimental conditions. The following figures show the results observed in a cat after breathing nitrogen, carbon dioxide, and carbon monoxide:

Atmosphere breathed	Reaction of blood $p(H)$	% of CO_2 in blood	Amplitude of respiration
Air	7.25	40	15
N_2	7.23	33	76
CO_2 (20 %)	7.2	57	84
CO	7.2	29	70

Marked changes in the reaction of the blood can be produced by the intravenous injection of lactic acid. The following figures show the effect of injecting 10 c.c. of lactic acid (1 %) into the jugular vein of a second cat:

	Reaction of blood $p(H)$	% of CO_2 in blood	Amplitude of respiration
Normal	7.39	34	9
During lactic acid dyspnœa	7.25	29	38
After recovery	7.29	31	9

Equally marked changes in the alkaline direction can be produced by the intravenous injection of 10 c.c. of Na_2CO_3 (10 %). The reactions of the blood etc. before and after the injection of this quantity of Na_2CO_3 into a rabbit were as follows:

	Reaction of blood $p(H)$	% of CO_2 in blood	Amplitude of respiration
Normal	7.27	37	13
After the injection of 10 c.c. Na_2CO_3 (10 %)	7.5	69	8

A comparison of these results shows that CO_2 and lack of oxygen (breathing N_2 or CO) produce marked increases in respiratory movement with very little change in the reaction of the blood whilst the intravenous injection of lactic acid and sodium carbonate produce great changes in the reaction of blood with small effects on the amplitude of respiratory movement.

Connexions of the enteric nerve cells. By J. N. LANGLEY.

Since nicotine paralyses the vagus fibres at their entrance into the stomach, it may be presumed that the vagus fibres are preganglionic, and end in the enteric nerve cells. The evidence that the sympathetic nerve fibres do not end in the enteric nerve cells is that its postganglionic fibres are not paralysed by nicotine. On this basis one nerve cell and only one would be on the course of a nervous impulse to the gut, as on that to other autonomic tissues. But the reactions of the gut are more complex than those of other tissues, and especially the peristaltic reaction as described by Bayliss and Starling. This, and the histological results of Dogiel, though these results have not been confirmed by Cajal, have led to the theory that sensory nerve cells are present in the enteric system, and to the theory of Bayliss and Starling that peristalsis is a co-ordinated reflex.

The nerve strand which the vagus sends to the greater part of the small intestine is small and the enteric nerve cells in the part supplied by it are in hundreds of thousands if not in millions. I do not, then, think that the vagus can run to them all. It seems not improbable that the later formed enteric cells lose direct connexion with the vagus, though remaining connected with what may be regarded as the mother cells, so that the connexions are essentially as in the diagram (Fig. 1).

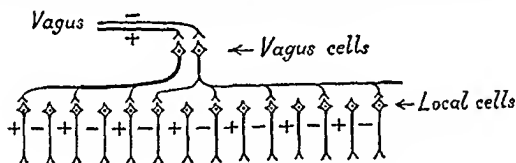


Fig. 1. + motor fibres; - inhibitory fibres.

With such connexion there are no sensory cells, and peristalsis would be the result of stimulation of the vagus cells, by the contents of the gut and by distention of the gut wall. On this scheme the connexions of the vagus with the gut would differ from that of other autonomic nerves in being tri-neuronic instead of bi-neuronic.

The theory that the enteric nerve cells are connected with the sympathetic as well as with the vagus depends upon embryological results (Kuntz, Miss Abel, E. Müller). The weak point of these is that the development of the nuclei traced from the central nervous system to the gut has not been followed late enough. They may be the nuclei of sheath cells of nerve fibres and not of nerve cells.

A Simple Perfusion Apparatus. By W. E. DIXON.

This perfusion apparatus consists of two pieces. One is an oven shown in Fig. 1, sufficiently large to hold all the working parts of the apparatus, the perfusing fluid and the organ perfused. It has the following internal dimensions, 25 ins. high, 17 ins. wide, and 12 ins. deep. It is provided with a 3 inch water jacket with an opening for filling and a tap for emptying. There is a hole at the top for a thermostat when this is required for very long experiments, though in practice we have found that with the very large water jacket and the complete felt covering provided

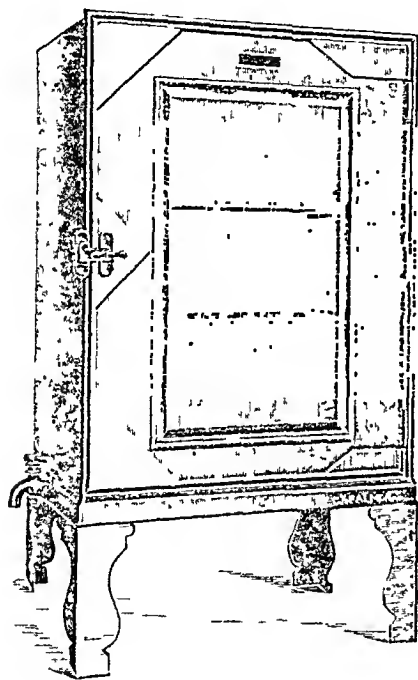


Fig. 1.

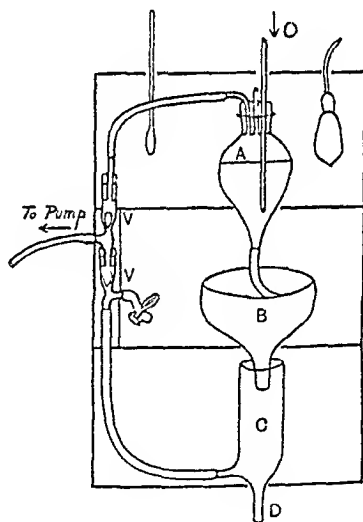


Fig. 2.

the temperature varies only very slightly within an hour or two, and by placing a small Bunsen beneath the oven it is easy to keep a constant temperature. Two narrow movable shelves with suitable holes through them trisect the oven (Fig. 2); on the upper is the flask *A* containing the perfusing fluid; this is provided with a rubber cork through which three holes pass, the first receiving the tube transmitting the venous return flow from the perfused organ; the second tube passes from without to the bottom of the flask, and is connected to the oxygen cylinder.

The third opening is to allow excess of oxygen to escape, the pressure of oxygen being regulated by a mercury valve. The flask tapers off below to a tube which is connected to the artery of the organ. The perfused organ is placed in a suitable retainer such as a Buchner funnel *B* and the fluid allowed to escape from the cut veins directly into the flask *C* or if

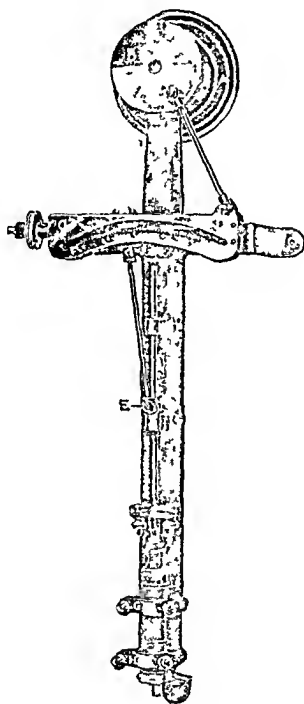


Fig 3

preferred, that is when exact rate of perfusion from minute to minute is required rather than metabolic changes, a cannula may be inserted into the vein and connected through a rubber cork to the receiving flask *C*. The circuit is completed by means of Bidie's valves worked by

a pump. Samples of fluid may be drawn off from the tap *D* as required. By connecting the retainer *C* to a volume recorder the volume of the air-space of *C* may be determined, *i.e.* the difference between the rate of filling and emptying. The object of the pump is to provide a very simple and rapid method of equalising these two.

The perfusion pump consists of an ordinary hypodermic syringe specially mounted to be worked mechanically from the laboratory shafting. The main feature of the pump is a variable stroke which can be very easily adjusted while it is working and the fact that the piston on all strokes comes to the bottom (outlet) end of the barrel and therefore the internal capacity remains constant.

In Fig. 3, *A* is the driving pulley having four V grooves for round band. *B* is a disc crank which moves the arm *CD* through a fixed arc on a pivot at *C*. On *CD* is a radial graduated slot having its centre at the joint *E*, when the arm is at its lowest position. Along this slot and moved by a screw having a milled head shown near *C* is a block which gives motion to the piston rod. Now it is obvious that when the arm *CD* is at its lowest position, the block can be moved along the slot without moving the piston of the pump, it follows that the further from the end *C* the block is moved the greater is the movement imparted to the piston on the upward stroke of *CD*.

The thrust of the pump can be read on the arc and if the internal diameter of the syringe is known the amount of fluid withdrawn from the receiving chamber *C* is known. In practice it is convenient to place a U tube containing mercury between the pump and valves to prevent escape of fluid to the pump. The ingenuity of the pump is due to the maker.

PROCEEDINGS
OF THE
PHYSIOLOGICAL SOCIETY,
July 8, 1922.

On the supposed relation of the sympathetic to muscle tone.
By K. UYENO.

It has been shown by Dusser de Barenne, Negrin y Lopez and v. Brucke, and by Rijnberk that extensor rigidity occurs in cats whether the sympathetic supply to the muscles is cut or not. Rijnberk¹ cut the abdominal sympathetic on one side and did not find any difference either in the extent or duration of the rigidity in the hind-limbs. Stimulation of the sympathetic has not been found to cause increase of tone in other cases, but apparently it has not been tried in decerebrate rigidity. In view of this and of the possibility that vaso-constriction might influence the tone I have tried the effect of stimulating the stellate ganglion and of applying nicotine to it.

The ganglion was exposed from the back and the sympathetic tied below it and cut. The branches to the heart were also cut. On lifting the ganglion by the ligature it could be stimulated without escape of current. This operation was made both before and after decerebration. In the former case the rigidity generally developed to nearly the same extent in both fore-limbs. In the latter case the rigidity diminished or was completely abolished during the operation, but it returned to about the previous degree some minutes after the operation. Sometimes the rigidity was slightly less in the fore-limb on the side of the exposed ganglion, this was certainly due to the sensory stimulation set up by the operation. To make the condition of the sensory stimulation due to the operation nearly the same on both sides, I exposed in one case the stellate ganglion on both sides and stimulated it on one side. In no case could I detect the slightest change in the rigidity of the fore-limb on the stimulated side.

¹ *Arch. neerlandaises* 7, p. 727, 1917.

In the case of nicotine the ganglion was exposed on both sides, and the sympathetic trunk was left intact, the heart branches were tied and cut on one side, lifting the thread, nicotine was brushed on both surfaces of the ganglion. No change in the rigidity was noticeable. I then extirpated the ganglion; the comparative rigidity of the two sides was unaltered and remained the same for several hours.

I conclude that neither stimulation of the stellate ganglion nor its paralysis by nicotine have any appreciable effect on decerebrate rigidity.

I wish to thank Prof. Langley for his kind suggestion about this work.

The influence of the depressor fibres of the vagus on the capillaries. By R. J. S. McDOWAL.

If in the cat, the blood-pressure is reduced by stimulation of the central end of the vagus (cardiac effects being excluded), the usual fall in pressure caused by the injection of histamine is greatly reduced or may be almost abolished. Striking results have been obtained, showing that the extent of the fall in blood-pressure due to a given dose (·01 mg.) of histamine which is rapidly recovered from, is inversely proportional to that from stimulation of the depressor fibres of the vagus. If the stimulation is carried out during a marked histamine fall, the action of the depressor is similarly much reduced, and may be seen only in the maintenance of the pressure at a low level without any further fall. Simultaneous stimulation and injection when the blood-pressure is at a normal level causes a larger fall than either singly.

Provided the anæsthetic and dosage are adequate the injection of histamine and such stimulation in a curarised cat both cause a fall in venous pressure.

The above results suggest that the depressor fibres of the vagus have a similar action to histamine which dilates the capillaries only (Dale and Richards) and consequently when one has lowered blood-pressure the other can no longer do so to its full extent. They also suggest, for the first time in mammals, the existence of a general capillary vaso-dilator mechanism under nervous control.

The formation of fat from protein. By E. W. AINLEY WALKER.

In view of present physiological opinion on the question whether fat can be formed from protein in the animal body, and our lack of conclusive information regarding the possible precursors of fat in vegetable

cells, it seemed desirable to study the question in relation to bacterial metabolism

Accordingly, several varieties of bacteria were cultivated in media rendered sugar free by fermentation. These media were prepared from "meat water" (got by boiling and filtering minced lean veal), peptone (Fairchild), and salt, or from peptone, salt, and tap water alone.

The amount of fat present in them was determined by means of controls. The peptone itself also yielded a little fat to ether extraction. The cultures and the controls were extracted with ether and the extracts saponified by boiling with known quantities of alcoholic potash, which were then titrated against deci normal hydrochloric acid.

The evidence obtained shows that more fat can be extracted from the cultures than from the controls, and suggests that bacteria can form fat from protein.

(The experiments were made in connection with work on the "Variability of bacteria" undertaken for the Medical Research Council.)

Bacterial products (P accessory factors) in relation to bacterial growth. By E W AINLEY WALKER

Bacteria are commonly grown on media which, from their method of preparation, are presumably free from "vitamines." Hence if they employ the so called accessory factors in their growth they must possess the power of synthesizing these substances. On the other hand, some of the more highly parasitic bacteria refuse to grow, or only grow with difficulty, on the ordinary culture media.

I find that the growth of bacteria of the latter class (*e.g.* *Gonococcus*) is rendered possible, or greatly improved, as the case may be, by the addition to media of a proportion of alcohol ether extracts of marmite or egg-yolk.

It is even more markedly assisted by the addition of fluids in which more robust bacteria have been allowed to grow and autolyse for a length of time.

The conclusion suggested is that, whereas most bacteria can produce for themselves any accessory factors which they require, the more delicate and more purely parasitic forms have lost this power by disuse, and that their obligatory parasitism is dependent on this fact.

(The observations were made in connection with work done for the Medical Research Council.)

Hæmorrhage and blood volume. By W. M. BAYLISS.

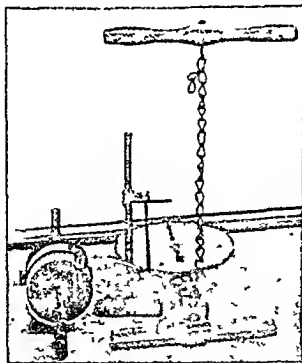
When an artificial fluid, such as gum-saline, is used to replace lost blood, it is plain that there is some limit to the hæmoglobin concentration below which the tissues must suffer from want of oxygen. In a series of experiments on anæsthetized cats, undertaken with the object of getting light on the question, I found that, provided that not more than 70 p.c. of the calculated blood volume was removed, it could be effectively replaced by gum-saline, so far as absence of any sign of respiratory or other failure was concerned; whereas a loss of anything over 30 p.c. was always fatal in the course of two to three hours, if not replaced. The volume of blood was taken as 6.2 p.c. of the body weight. If 7.5 p.c. be assumed, 70 p.c. loss of blood becomes 57 p.c. Observations were not made at a later date after injection than six to seven hours, but Barthélémy reports in dogs a similar limit for "definitive" survival. In my experiments, if more than that mentioned was removed, the animals did not survive more than three hours, even if the volume was made up by gum-saline or glucose-plasma. Various observations by Bazett on cases of toxæmia in man gave 25 p.c. of the normal hæmoglobin content as the lowest limit for continued existence. Robertson and Bock found the same limit as regards ultimate regeneration of corpuscles. This corresponds to a reduction of the corpuscles to 12.5 p.c. of the blood. In the cat, a hæmorrhage of 70 p.c. would reduce them to 12 p.c., so that the results agree. In some of my experiments, the dilution of the blood was determined. Cats with 14.6 and 12.9 p.c. of the normal hæmoglobin showed no signs of failure; those with 10.7, 9.45 and 6.1 died in the course of two hours or less. It is surprising that the normal percentage of hæmoglobin is so much in excess of that required for supply of oxygen in rest, so that provided the normal blood volume is kept up, a hæmorrhage amounting to some three or four litres in man can be replaced by gum-saline. The explanation of the importance of the volume of the blood lies in the experiments of Gesell, who found that the dilution was far more than compensated by the increased irrigation, or "nutrient flow."

In a paper by Yandell Henderson and H. W. Haggard in the *Journ. Amer. Med. Ass.* for March 11th, 1922, of which an abstract is given in these *Proceedings* for February 18th last, the conclusion is arrived at that in hæmorrhage it is the hæmoglobin that is the decisive factor and not the blood volume. Hence, no artificial fluid can supply the place of blood. Their results are to be accounted for by the excessive

degree of hæmorrhage and so far agree with mine. In the experiment with gum-saline of which a detailed account is given, 80 p c. of the blood was removed, on a basis of 7.3 p.c.; if 6.2 p.c. be assumed, it amounted to 94 p.o., although doubtless some replacement from the tissues occurred. Their method of experiment was fallacious also in that blood-pressure was taken as criterion for the amount to be removed, not blood volume. Moreover, their table shows that of the cases which recovered when the blood was replaced by gum-saline, 5.1 p.c. of the body weight could be removed; whereas if this was not done, only 3.7 p.c. could be removed.

The influence of rest pauses and changes of posture on the capacity for muscular work. By H M VERNON

The dynamometer used consisted of a 500 lb spring balance set up as in the figure. The subject usually stood on the T-piece when pulling, but a foot rest was provided so that he could also pull when sitting. The muscular contractions were practically isometric.



The dynamometer was first used for testing the maximum strength of pull or weight-lifting power. Taking the lifting power at floor level as 100, it was found that it gradually sank to a minimum value of 74 to 82 at a height of 15 inches above the floor, and then increased till it attained a maximum of 118 to 130 at a height 28 inches above floor level. At greater heights it fell rapidly. The weight-lifting power is only 2 p.c. less

when the subject stands on one leg instead of two, but it is only just over half as great when he uses one hand instead of two.

When the dynamometer is pulled at regular intervals, the initial strength of pull falls rapidly for about four minutes, and then for a long time keeps at a nearly constant level. The height of this depends on the frequency of the rhythm, being 53 p.c. on the initial height when pulls were made every $1\frac{1}{2}$ seconds, and 85 p.c. when made every 4 seconds.

In most of the experiments on rest pauses, the duration of the rest periods was to that of the work periods as 1 to 4, or as 2 to 3. If the subject of experiment remained motionless during the rest period, the total work done was always less (sometimes 27 p.c. less) than when no rests whatever were taken, but if the subject gently bent his shoulders back and moved his arms about, these changes of posture caused a tremendous reduction in the fatigue effect. The total work done was 2 to 14 p.c. greater than when no rests were taken, and the strength of pull remained at a constant level for as long as the experiment was continued (*e.g.* 88 minutes). When no changes of posture were made during the rests, the strength of pull continued to fall throughout the experiment.

The posture effect is probably due in chief part to the influence of the postural changes on the circulation, as was suggested by the following experiments. (1) It was found that if the strength of pull were determined (*a*) when standing, (*b*) when sitting, and (*c*) when alternately standing and sitting (alternations being made at 40 seconds, 2 minute or 5 minute intervals), the average strength of pull in (*c*) was 6 to 15 p.c. greater than the average of (*a*) and (*b*). Presumably the alternate standing and sitting movements, being of a somewhat different character, promoted the circulation. (2) If an occasional pull were made during the rest pauses of a "posture unchanged" experiment, the fatigue effect was considerably diminished in spite of the extra work done, as each pull of itself promotes the circulation. (3) If the shoulders were bent back about $2\frac{1}{2}$ inches during each individual pull, instead of being kept immoveable, the fatigue effect was greatly diminished, and the strength of pull was almost as great when no postural changes were made during the rest pauses as when they were made. (4) If a hot bath were taken 20 minutes before an experiment, the fatigue effect was very much more pronounced, presumably because some of the usual blood supply was diverted from the muscles to the skin.

Rest pauses of the same relative duration to the work periods were taken at intervals of $2\frac{1}{2}$ seconds, 10 seconds, 40 seconds, 2 minutes and

5 minutes. The capacity for work is but little affected by the length of the interval, but probably it is slightly greater with 2 minute and 5 minute intervals than with the shorter ones.

The pulse showed no greater acceleration in the experiments when the posture was changed during rest pauses than when it was not changed, in spite of the fact that 22 per cent more work was being done.

A full account of these investigations will be published shortly.

The effect of atmospheric cooling power on the pulse rate and on the efficiency during muscular exercise. By

LEONARD HILL and JAMES ARGYLL CAMPBELL

Most of these observations were carried out on a trained subject (C. P.), who has now been employed in such experiments for nearly two years. Schuster's modification of Martin's bicycle ergometer was used whilst the respiratory exchange was estimated by the Douglas Haldane method, as recommended by Cathcart (*Journ. R.A.M.C.* Nov. 1918).

The experiments lasted 15 minutes, the rate of pedalling was about 70 revolutions per minute, this rate being the most suitable, as pointed out by Benedict and Cathcart ("Muscular Work," 1913). In calculating the gross and nett efficiencies we have followed the usual method.

The sample of expired air was taken during the last three minutes in each experiment, care being taken that the rate of pedalling and the kilogrammeters of work done per minute were constant throughout the 15 minutes. Any short calorimetric method which neglects this important factor must obviously give only approximate results.

The atmospheric cooling power was measured by Hill's kata thermometer. Cooling powers varied from 3.9 to 11.2 millicalories per sq. cm. per sec., the former being obtained in a warm room, and the latter in a cool tunnel. The conditions in this warm room have been observed in some workshops.

It was found that under the conditions specified the cooling power had no effect on the efficiency. Thus, in one experiment under a cooling power of 5.0 and working 507 kgm. per min. the gross efficiency was 16.1, and the nett efficiency 19.3, whilst in another experiment, under a cooling power of 10.8 (twice as cool) and working 515 kgm. per min., we found practically the same figures for the efficiencies, namely 16.9 and 20.0 respectively. On the other hand, the pulse rate was quite different in these two experiments—being 156 under the cooling power of 5.0,

and 120 under the cooling power of 10.8. The personal feelings of the subject as regards comfort were, of course, much more pleasant under the higher cooling power (see Table for other experiments).

Apparently then, in these short experiments, the cooler conditions greatly relieved the heart, although the efficiency of muscular contraction was not affected. This is an indication of the value of cool conditions during work, for fatigue of the heart will shorten the working period of men in warm surroundings, and thus lower the total working efficiency of the day.

*Experiments of 15 min. duration (subject, C. P.).
(Ordinary clothes worn.)*

Pulse	Dry kata- thermometer cooling power	Work Kgm. per min.	Efficiency p.c.	
			Gross	Nett
120	10.8	515	16.9	20.0
120	10.3	509	15.6	18.5
125	10.9	507	—	—
150	7.5	514	—	—
156	5.0	507	16.1	19.3
104	10.9	399	15.6	—
108	10.3	406	16.9	21.6
108	10.8	407	15.6	19.6
108	11.2	407	14.8	18.3
120	3.9	414	15.1	18.4
132	5.0	408	15.9	20.0

**An investigation into the physiological significance of the
"40 mm. mercury test."** By MARTIN FLACK and H. L. BURTON.

This test has been extensively employed in the Royal Air Force as one of the tests for Physical Efficiency, and is popularly known as the "Endurance" or "Fatigue" test. The test is performed as follows:

With the nose clipped the subject is directed to empty the lungs as completely as possible, inhale fully, blow the mercury in the U-tube to the height of 40 mm. and maintain it there without breathing as long as possible, the cheeks and lips being supported by the hand in such a manner that they take no part in the process. From the other wrist the rate of the pulse is recorded during periods of five seconds. In a previous communication¹ details have been given in regard to length of time and

¹ *Medical Research Council Special Report Series*, No. 53, p. 93.

types of pulse response observed with this test, it being shown that these factors vary according to the fitness, as judged by the executive authorities, of the subject for flying duties.

To ascertain more fully the physiological significance of the test the results under varying conditions have been compared with those obtained when the breath is held after full expiration and full inspiration, the systolic and diastolic pressures being measured at intervals during the test. The tests have in common the fact that in each case the test is performed upon the full vital capacity of the individual, they differ in that in the 40 mm. test the expiratory muscles are actively engaged in raising the intra-abdominal pressure.

It has been found that in the 40 mm. test as in breath-holding test the time during which the test can be performed is extended by preliminary forced breathing or breathing oxygen and shortened by breathing carbon dioxide or air deficient in oxygen. It has also been found that some individuals are more susceptible to these changes than others. In the fit subject it requires a considerably greater diminution of the partial pressure of oxygen in the alveolar air to induce symptoms of respiratory instability (violent desire to breathe, etc.) and signs of circulatory embarrassment (quickened heart rate, increased blood-pressure, congestion of face) than it does in the subject who is known to be suffering from the effects of fatigue or any condition lowering general efficiency. Likewise the fit subject can tolerate a larger percentage of carbon dioxide in the alveolar air before manifesting distress. Both tests therefore may be taken as affording indication of the ventilation capacity of the individual and of his susceptibility, as manifested through the action of the medullary centres, to increase of CO_2 and of diminution of oxygen in the alveolar air.

In addition it would appear that, through the nature of the pulse response, the 40 mm. Hg test may be regarded as affording information as to the distribution of the blood within the circulation, giving thereby a means of estimating the degree of "abdominal pooling" of the blood within the individual. When performed to an average degree of discomfort, there is, in the fit subject, little or no alteration of the rate of the pulse, and the arterial pressures are not greatly raised (30-40 mm. Hg). In the less fit, however, comparison of the results of the tests shows that, with the 40 mm. mercury test, the arterial pressures are more quickly raised and to a greater height, while the pulse rate rapidly increases, an increase maintained when the total rise of pressure is not abnormally high, but succeeded by a marked and often abrupt fall of pulse rate when

the arterial pressure is increased to a high point which appears to be constant for each individual.

The following explanation is suggested: The abdominal effort necessary to sustain the column of mercury increases the intra-abdominal pressure. In subjects, with a tendency to abdominal pooling, this pressure drives blood onwards to the right heart, causing thereby an increase of heart rate, together with an early marked rise in arterial pressure. In subjects where this pooling is unduly great the systolic pressure becomes so increased that, following the acceleration due to an increased supply of blood to the right side of the heart, there supervenes reflexly through the action of the vagus a marked slowing mainly owing to the stimulation of the depressor nerve endings in the aortic arch.

These facts are supported by the following figures taken from selected cases:

Subject	Time in secs.	Pulse response in 5 second periods	Arterial pressures	S. P.	D. P.	Remarks
H. S. C. S.	105	6-7, 7, 7, 6, 6, 6, 6, 7, 6, 7 All 6's	Before	122	76	B. H. Test on air. Fit subject trying his utmost.
			20-25"	136	88	
			60-65"	164	112	
			Breakdown	178	—	
H. S. C. S.	85	6-6, 7, 7, 7, 7, 7, 8, 8, 7, 7, 7, 8, 6, 6, 6, 5, 6	Before	122	80	40 mm. Test on air. Ditto.
			20-25"	148	108	
			60-65"	178	136	
			Breakdown	180	—	
M. A. C.	63	6-7, 9, 8, 7, 7, 8, 8, 7, 6, 6, 5, 5	Before	164	96	B. H. Test on air. Subject, recovering from influenza, trying his utmost.
			20-25"	190	116	
			50-55"	242	138	
M. A. C.	60	6-8, 10, 12, 11, 11, 11, 9, 9, 8, 6, 6, 5	Before	162	94	40 mm. Test on air. Ditto.
			15-20"	202	120	
			50-60"	256	142	
H. A. B.	32	7-9, 9, 9, 8, 7, 7	Before	154	70	B. H. Test on air. Fatigued subject.
			15-20"	174	92	
			Breakdown	200	—	
H. A. B.	38	7-10, 13, 13, 12, 12, 12, 12	Before	156	72	40 mm. Test on air. Ditto.
			15-20"	212	122	
			Breakdown	240	—	

The neutrality of blood. By J. MELLANBY and C. C. WOOD.
(*Preliminary communication.*)

The preservation of the neutrality of blood under different tensions of carbon dioxide is due to the opposite effects of this gas on the corpuscles and plasma. The corpuscles of blood, partially freed from CO₂ and suspended in .85 p.c. NaCl, have a reaction which lies on the acidic side of neutrality. When exposed to alveolar air these corpuscles take up a considerable quantity of CO₂ and the reaction of the fluid moves

towards the neutral point. On the other hand, serum from the same blood is alkaline in reaction, and on exposure to alveolar air becomes more acidic.

Defibrinated blood was partially freed from CO_2 by CO_2 free air and separated into corpuscles and serum by prolonged spinning in a powerful centrifuge. The corpuscles were suspended in .85 p.c. NaCl. The reaction and quantities of CO_2 carried by (a) the suspension of corpuscles in .85 p.c. NaCl, (b) the serum, and (c) the blood, before and after putting into equilibrium with alveolar air, were as follows:

(a) *Corpuscles:*

	Reaction $p(\text{H})$	CO_2 content
Before alveolating	6.4 p.c.	5.5 p.c.
After ,,	7.1	50

(b) *Serum:*

Before alveolating	7.3	23
After ,,	6.9	48

(c) *Blood:*

Before alveolating	7.2	17
After ,,	7.1	65.5

The apparent paradoxical effect of CO_2 on the red cells offers a simple explanation for the approximate neutrality of blood under varying tensions of CO_2 . Possibly it also explains the divergence which exists between the calculated reaction of blood, based on the ratio of the free and combined CO_2 contained in it, and that actually observed by direct experiment.

Pressure and stretching as independent factors in the production of œsophageal pain. By W. W. PAYNE and E. P. POULTON.

Pressure. This was investigated by means of a 20 c.c. cylindrical rubber bag tied on to the end of a gum elastic œsophageal catheter with a syringe attached to the other end. The bag was encased completely in linen, and the whole system filled with water. The catheter was passed into the œsophagus of one or other of us. The bag could be tightly filled with water, and owing to its linen cover was practically incompressible. A second uncovered rubber bag tied on to the end of a rubber catheter and containing air, was placed in the œsophagus just above the water bag. It was connected to a water manometer; and the other end of the latter to a Brodie's bellows, so that contraction and relaxation of the

œsophagus were recorded on a kymograph. On filling up the water bag tightly continuous substernal pain was felt which became worse after swallowing; the pains coincided with continuous contractions of the œsophagus.

Our explanation is that pressure exerted on the nerves of the œsophagus by the muscle contracting against the incompressible bag is the cause of the pain.

Stretching. In this experiment, the air bag connected with the recording apparatus was alone used. It could be filled to any desired extent with air. Periodic substernal pain was experienced, and there were also peristaltic movements of the œsophagus. In this case the pain was felt during relaxation and was abolished by the contraction.

Our explanation is that in the former case, the bag stretched the œsophagus and so pulled on its nerves; in the latter, the stretching was relieved while the pressure in the bag was not sufficient to stimulate the nerves by compression.

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